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Sampling stream invertebrates using electroshocking techniques: implications for basic and applied research

Brad W. Taylor, Angus R. McIntosh, and Barbara L. Peckarsky

Abstract: We present a new technique using electrofishing equipment to collect and quantitatively sample stream invertebrates. We used an electrofishing machine with a small anode to produce a localized field of pulsed direct current to induce invertebrate drift. We quickly obtained large numbers of live invertebrates for experiments by passing the anode over the stream bottom upstream of sampling nets. We compared the results of five techniques: (*i*) electroshocking inside a modified Hess sampler, (*ii*) repeated electroshocking over a large area to estimate population size by depletion, (*iii*) traditional Surber, (*iv*) Hess, and (*v*) individual stone sampling. Electroshocking techniques provided estimates of invertebrate density comparable with those of traditional sampling techniques. The electroshocking depletion method that sampled a large area provided higher measures of Ephemeroptera, Plecoptera, and Trichoptera richness. Hess and area-restricted electrobug methods had similar density and diversity estimates, whereas the Surber sampler provided low density estimates, especially for mobile taxa. Density estimates from individual stones were inflated, were biased for mayflies, and had low richness. Samples taken with the electroshocking method were processed 40% faster because these samples contained little detritus. Electroshocking techniques can provide accurate estimates of population size and diversity, minimize disturbance to benthic habitats, and reduce processing time.

Résumé : Nous présentons une nouvelle méthode de récolte et d'échantillonnage quantitatif des invertébrés des cours d'eau à l'aide d'un appareillage de pêche électrique. Nous utilisons un appareil avec une petite anode qui produit un champ restreint de courant direct à impulsions pour provoquer la dérive des invertébrés. Nous pouvons ainsi obtenir rapidement de grandes quantités d'invertébrés vivants pour usage expérimental en passant l'anode au-dessus du fond en amont de filets de récolte. Nous avons comparé l'efficacité de cinq techniques: (*i*) la pêche électrique dans un échantillonneur de Hess modifié, (*ii*) la pêche électrique répétée sur une grande surface pour estimer la population par retraits successifs, (*iii*) l'utilisation de l'échantillonneur de Surber classique, (*iv*) l'utilisation de l'échantillonneur de Hess et (*v*) la récolte manuelle sur des pierres individuelles. Les méthodes de pêche électrique fournissent des estimés de densité des invertébrés comparables à ceux des méthodes traditionnelles. La méthode d'estimation avec retraits par pêche électrique sur une grande surface fournit des estimations plus élevées des densités (richesse) des éphéméroptères, des plécoptères et des trichoptères. La méthode de Hess et la pêche électrique sur surface réduite donnent des estimations similaires de densité et de diversité, alors que la méthode de Surber fournit des estimations basses, particulièrement des taxons mobiles. Les estimations obtenues par récolte sur les pierres individuelles sont trop élevées; les éphéméroptères y sont surreprésentés et la richesse en espèces est basse. Le traitement des échantillons obtenus par pêche électrique est de 40% plus rapide, parce qu'ils contiennent peu de détritus. Les méthodes basées sur la pêche électrique peuvent donc fournir des estimations précises de la densité et de la diversité des populations, minimiser la perturbation des habitats benthiques et réduire le temps de manipulation des échantillons.

[Traduit par la Rédaction]

Introduction

Sampling benthic invertebrates is a challenging task that confronts many studies of running waters. Although numerous papers and reviews have examined sampling of stream and riverine benthic communities (Cummins 1962; Merritt et al. 1984; Peckarsky 1984), accurate and efficient collecting and processing of invertebrate samples remain difficult. Problems arise because no sampling device is suitable for all types of habitat (e.g., dependence on flow or sampler area).

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In addition, the patchy distribution of invertebrates and attributes of the organisms (mobile or attached) can result in underestimates of population densities and (or) sampling bias. Moreover, benthic samples taken using current methods are usually labor intensive to process.

Traditional methods for sampling benthic invertebrates from streams rely on physically disturbing the substrate and collecting invertebrates in a net held downstream (Surber 1937; Hess 1941; Frost et al. 1971). Invertebrates are usually preserved along with detritus for picking and sorting in the laboratory or are sorted in the field if live specimens are desired. Since invertebrates are often associated with detritus (Cuffney and Wallace 1987) and interstitial spaces of the stream bottom (Reice 1980), these techniques have proven effective at sampling those habitats. However, the large amounts of detritus included in these samples make them time-consuming and difficult to process. Further, most traditional sampling devices only sample a small area of the substrate (Surber, 0.09 m^2; Hess, 0.09 m^2; D-net, undefined). Given the patchy distribution of invertebrates in streams (Downing 1979; Lancaster et al. 1991), a large number of samples must be taken to minimize the variance in measures of abundance and accurately estimate population size and (or) the diversity of a community (Needham and Usinger 1956; Allan 1982; Morin 1985). These estimates are often compromised by time and financial constraints that reduce the optimal number of samples that can be taken (Sheldon 1979; Ferraro et al. 1989).

To advance sampling of benthic invertebrates, a method is required that removes invertebrates effectively from the substrata while minimizing the amount of debris collected and that allows a larger area to be sampled, integrating more habitats and reducing variance among samples. Electrical current (electroshocking) to stun and capture freshwater fish has been used for many decades (Cowx 1983; Hayes and Baird 1996), but the application of this technique to sample benthic invertebrates is relatively new. Recently, studies have explored the use of electroshocking to sample decapods (Penczak and Rodriguez 1990; Fièvet et al. 1996; Rabeni et al. 1997) and in association with freeze-core sampling of hypoheic stream fauna (Bretschko 1990). Electroshocking has also been used to collect large numbers of mayfly larvae for experiments (McIntosh and Townsend 1994). Other studies have examined the indirect effects of electroshocking for fish on invertebrate density and drift in a stream reach (Elliot and Bagenal 1972; Fowles 1975; Bisson 1976). In all of these studies, electroshocking induced drift of invertebrate taxa with no physical disturbance to the stream bottom. Thus, we hypothesized that a technique using electroshocking would be effective for making quantitative estimates of invertebrate abundance with improved sample processing efficiency because the amount of detritus collected would be reduced.

Here, we describe new methods for quantitatively sampling invertebrates from streams that use standard electrofishing equipment to induce invertebrates to drift into capture nets. Two methods were developed: one requires electroshocking inside a 0.09-m^2 modified Hess sampler (area-restricted electrobug) and the other involves repeated shocking to deplete invertebrate populations from a large area (2–4 m^2) delineated by upstream and downstream nets (depletion by electroshocking). First, we compared differences in density estimates, efficiency, and size selectivity between the electroshocking technique and other methods (e.g., Surber, Hess, and individual stones). To determine the effects of electricity on invertebrates, we compared the drift behavior, growth rate, and mortality of a common mayfly, *Baetis bicaudatus*, collected using electroshocking with those of individuals collected with a D-net. Next, we compared estimates of biotic indices used in biomonitoring programs between electroshocking techniques and traditional sampling methods. Finally, we compared the processing time (picking and sorting) for samples collected with the electrobug technique and the traditional Surber technique.

Materials and methods

We sampled invertebrates from the East River and tributaries in the vicinity of the Rocky Mountain Biological Laboratory, a high-altitude (2945 m) field station located in Gunnison County, Colorado, U.S.A. Streams at this elevation are high-gradient, cobble-bottom streams, ranging in conductivity from 126 to 270 $\mu S \cdot cm^{-1}$, with mean summer temperatures of 5–13°C. Streams in the East River watershed range in size from first-order streams with an average width of 1.7 m and summer discharge of 2.8 $L \cdot s^{-1}$ to third-order streams with average width and discharge of 9.3 m and 770 $L \cdot s^{-1}$, respectively (Peckarsky et al. 2000, 2001). Invertebrate communities in these streams are dominated by Ephemeroptera (mayflies), Plecoptera (stoneflies), Diptera (true flies), and Trichoptera (caddisflies) (Peckarsky 1991).

Sampling procedures

Five quantitative sampling procedures were used: (*i*) electroshocking within a Hess-type sampler (area-restricted electrobug sampling), (*ii*) depletion electrobug (sampling using electroshocking equipment over a large area), (*iii*) Surber sampling, (*iv*) Hess sampling, and (*v*) individual stone sampling. For both electroshocking techniques, we used a Smith–Root model 15-C backpack-style electroshocker to produce a pulsed direct current (DC) through the water. We replaced the standard 28-cm-diameter electrode (anode) with a 15-cm electrode (Smith–Root part No. APA83-6), which produced a stronger current over a smaller area. Effective voltage requirements were similar to those used for electrofishing brook trout (*Salvelinus fontinalis*) in these streams, ranging from 500 to 700 V DC depending on the conductivity. Although the Smith–Root electroshocker has a variety of frequencies and pulse widths, we found that the standard setting recommended for salmonids was satisfactory (60–80 pulses $\cdot s^{-1}$ and a pulse width of 6 ms).

In 1997, we compared five area-restricted electrobug samples and five Surber samples among three different streams in the East River watershed. In 1999, we compared five area-restricted electrobug, three depletion electrobug, five Surber, five Hess, and five individual stone samples from only one stream. Because the electroshocking methods induced drift of invertebrates, we took Surber, individual stones, and Hess samples before the electroshocking samples. Samples were stained with Rose Bengal and preserved in 95% ethanol. We classified substrate size (Wentworth 1922) for 10 randomly selected substrate particles in each sample area as a potential covariate with invertebrate density.

We constructed an area-restricted Hess (or Neil) type sampler with the same dimensions (30.5 × 30.5 cm) and area (0.09 m^2) as a Surber sampler but with clear plastic (Lexan®) sides and 280-μm Nitex® mesh on the upstream end. A U-shaped aluminum slot on

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the downstream end enabled a Wildco® 30 × 25 cm drift net (202 µm) to be inserted and removed from the sampler.

To sample invertebrates using the area-restricted electrobug sampler, we randomly selected five locations in each of three streams in 1997 and one stream in 1999. Holding the sampler in place while wearing insulated rubber gloves, we electroshocked close to, but did not disturb, the substrate within the sample area for 90 s. We created an electrical gradient from the area being sampled to the capture net by placing the anode inside the sampler and the cathode immediately downstream of the sampler. In 1999, we determined the efficiency of this sampler in one stream by removing the drift net after sampling, inserting a new drift net, and then disturbing the substrate by hand for another 90 s.

In 1999, for depletion sampling by electroshocking, we placed 2-m-wide × 1-m-high block nets (280-µm Nitex® mesh) at the upstream and downstream ends of each stream to isolate a large area (2–4 m^2) for sampling. The top and bottom edges of nets were pleated to create a bag shape, and lead weights were attached to the bottom. Nets were anchored in place with wooden poles fitted through sleeves on both sides, and three passes with the electroshocking unit were made. We stood on the streambank while shocking and did not disturb the substrate with the electrode. The cathode was placed below the downstream net to establish an electrical gradient from the anode to the cathode. Three width measurements and the length of the sample area were used to determine the area sampled. The size of 20 substrate particles within the sample area was also recorded (Wentworth 1922).

The efficiency was estimated using the equations derived by Zippin (1956, 1958). As an empirical test of the maximum likelihood functions used to determine the probability of capture during one pass, we also took five Hess samples from the stream reach after three passes had been made with the electroshocker to determine the number of invertebrates remaining after three passes.

In 1997 and 1999, we randomly selected five locations in each stream and disturbed the substrate by hand within the frame of a standard Surber sampler (0.09 m^2, 202-µm mesh) for 90 s. In 1999, we determined the efficiency of the Surber sampler in one stream by taking another Surber sample from the exact same location.

In 1999, we used the Hess sampler without electricity (Fig. 1) to sample invertebrates in one stream by physically disturbing the substrate by hand within the sampler area for 90 s. In 1999, we determined the efficiency of the Hess sampler in one stream by removing the drift net after the first sample, inserting a new net, and disturbing the substratum again for 90 s.

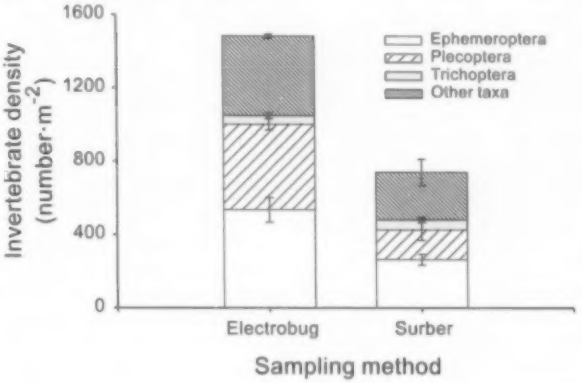
In 1999, invertebrate densities from individual stones were estimated in one stream by randomly selecting five stones (10–15 cm), placing a net (250 µm) directly downstream, and quickly lifting the stone into the sample net. Stone area was estimated by tracing the outside perimeter of each rock onto a clear acetate sheet. We used a digitizer to determine the two-dimensional surface area of each stone based on the perimeter tracing.

In 1999, to determine if the electroshocking technique was size selective, we measured head capsule widths of 120 mayfly (*Baetis bicaudatus*, Ephemeroptera: Baetidae) larvae (the most common invertebrate) from five different Surber and five area-restricted electrobug samples from one stream.

To determine if processing time differed between samples collected using the area-restricted electroshocking technique and Surber sampler, samples were emptied into picking trays in the laboratory and the time to sort and identify taxa was recorded. The same person processed all samples. The time to process a sample was divided by the total number of invertebrates in each sample to obtain the number of minutes to process 100 individuals.

To determine if there were any detrimental effects of electroshocking on invertebrates, we compared survival, growth, development, and drift behavior of *B. bicaudatus* collected using electroshocking and conventional kick sampling techniques. Larvae were collected from the East River by physically disturbing the substrate by hand and capturing *Baetis* with a D-net (500-µm mesh size) and with the electroshocker and D-net held downstream to collect invertebrates using electricity. We reared and observed *Baetis* in circular flow-through tanks (Peckarsky and Cowan 1991) with 15 individuals (stage III, summer generation, $n = 10$ tanks per treatment). Treatments were randomly assigned to tanks, and rocks with algae were placed in the tanks to provide food resources. We determined the initial size of a subsample of *Baetis* collected by drying individuals at 60°C for 24 h and then weighing them on a Cahn C-31® microbalance. Final size of all individuals was obtained similarly. Mortality was determined by counting the number of *Baetis* remaining after 7 days, corrected for the number that emerged, which was observed daily. To examine the possible effects of electroshocking on drift behavior, we counted the number drifting in each tank for 60 s during the night (20:00 mountain daylight savings time) on the evening after they were collected.

Fig. 1. Invertebrate density (mean ± 1 SE) for the area-restricted electrobug and Surber sampling techniques. Means are for three streams sampled in 1997.



Statistical analyses

Comparisons between area-restricted electrobug and Surber samples taken in 1997 were performed using a mixed-model analysis of variance (MANOVA) with stream as a random effect and sampling method as a fixed effect tested using the interaction term sampling method × stream as the denominator for sampling method. We tested effects of sampling method on multiple independent response variables using a MANOVA and individual ANOVA when the MANOVA was significant. Benthic densities were natural log transformed to normalize the data. Substrate size did not differ among sampling methods (ANOVA, $F = 1.02$, $P > 0.05$) and therefore was not included in analyses. Size–frequency distributions of *Baetis* were compared using a Kolmogorov–Smirnov two-sample test.

The patchy distribution and subsequent high variability in density estimates are one of the main problems associated with sampling stream invertebrates. Although we took samples randomly, it was also not possible to sample the exact same areas with each method. As a result, the statistical differences or similarities among methods may have been due to spatial differences in habitats sampled. We addressed this issue by performing a Levene (1960) test to assess homogeneity of variance among sampling methods.

To calculate mean densities, standard error of the mean, and efficiency for the depletion sampling technique, we used the maximum likelihood model developed by Zippin (1956, 1958). If a taxon was captured in only one out of the three passes, the Zippin

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model was not applicable and densities were determined by assuming that density equaled the number caught in one pass. When the number of invertebrates caught in previous passes was less than that in subsequent passes, density was estimated by multiplying the total number caught by the density estimate for the taxonomic group with the highest variance divided by the total number caught for that group. The difference in sample processing time between Surber and area-restricted electrobug samples was analyzed using Student's t test. Differences in survival, growth rate, development, and drift behavior of *Baetis* collected with and without electroshocking were analyzed using MANOVA after arcsine square root transforming the percent survival and emergence data and square root transforming the drift data. All statistical analyses were performed using SAS (SAS Institute Inc. 1989).

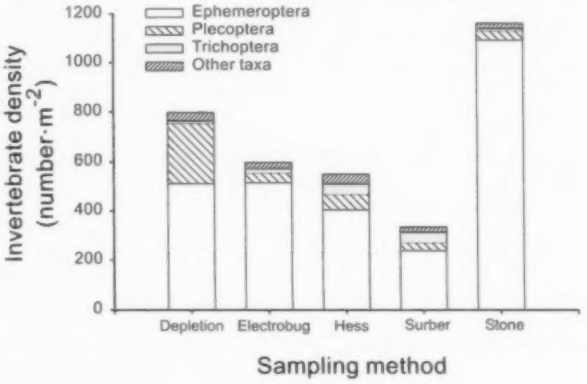
The number of individuals in a sample increases the probability of encounter for a given species; we standardized for abundance levels before comparing richness among techniques (Sanders 1968; Hurlbert 1971; Gotelli and Graves 1996). Each sampling technique was standardized to a common number of individuals, that is, we "rarefied" samples to a common abundance level (Hurlbert 1971; Simberloff 1972) for both total taxa richness and richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT richness; Lenat 1983) using EcoSim (Gotelli and Entsminger 1999). EcoSim uses Monte Carlo methods similar to rarefaction to estimate the expected richness for a given number of individuals (abundance level) drawn randomly from a sample (Gotelli and Graves 1996). We combined the replicates for each method within a stream and repeated the randomizations 100 times, which generated an average expected richness and confidence limits. Confidence intervals were used to compare differences among sampling techniques.

Results

There were significant differences in densities of invertebrates sampled by the area-restricted electrobug and Surber sampling techniques among the three streams sampled in 1997 (MANOVA, Wilk's λ, $F = 27.20$, $P = 0.0349$) (Fig. 1). These differences were attributed to low density estimates for Ephemeroptera ($F = 48.06$, $P = 0.0202$) and Plecoptera ($F = 18.22$, $P = 0.0409$) for the Surber method. There were no significant differences in density estimates for Trichoptera ($F = 1.33$, $P = 0.3681$) or "other taxa" ($F = 2.39$, $P = 0.2621$) between techniques. Differences between area-restricted electrobug and Surber sampling methods were not a function of variation in performance among streams, as indicated by the nonsignificant interaction between sampling method and stream (MANOVA, Wilk's λ, $F = 1.36$, $P = 0.2406$). In addition, the Levene (1960) test for homogeneity of variances among sampling methods within each stream was not significant ($P > 0.05$ for all taxonomic groups), indicating that the variances were equal among sampling techniques.

There were significant differences among the area-restricted electrobug, depletion electrobug, Surber, Hess, and individual stone sampling methods for estimates of total invertebrate density within one stream in 1999 (MANOVA, Wilk's λ, $F = 4.05$, $P < 0.0001$). Samples taken from individual stones had the highest density estimates with the greatest variability (Fig. 2). The traditional Surber technique gave the lowest density estimates, but this difference was only significantly different from the stone and depletion sampling methods. Examination of individual ANOVAs for each taxonomic group revealed that the high-density estimate for individual stone samples was driven by high estimates of Ephemeroptera ($F = 9.42$, $P < 0.0003$). Area-restricted electrobug and Hess sampling methods produced intermediate density estimates and were not significantly different from any of the other sampling methods (Tukey's, $P > 0.05$). Ephemeroptera were the most abundant group across all sampling methods. However, Surber samples gave the lowest density estimates for Ephemeroptera compared with all other sampling methods ($P < 0.05$). The Plecoptera were the second most common group (Fig. 2), and densities were statistically different among the five methods ($F = 2.98$, $P < 0.0474$) because of their abundance in depletion samples (Tukey's, $P < 0.05$). Densities of Trichoptera were significantly different among methods ($F = 4.46$, $P = 0.0112$), with Hess and Surber containing more than individual stone samples (Tukey's, $P < 0.05$). There was no statistical difference in densities of "other taxa" among the five sampling methods ($F = 1.05$, $P = 0.4089$).

Fig. 2. Invertebrate density (mean ± 1 SE) for the electroshocking depletion, area-restricted electrobug, Hess, Surber, and individual stone sampling techniques. Data are from one stream sampled in 1999. Error bars are omitted for clarity.



The Levene (1960) test for homogeneity of variance indicated that the variances were equal for total invertebrate density ($F = 0.94$, $P = 0.4634$) among the five sampling methods. Examination of the homogeneity of variance for the individual taxonomic groups revealed that for Ephemeroptera and Trichoptera, variances were equal among sampling methods ($P > 0.05$) but unequal for Plecoptera ($F = 11.05$, $P = 0.0001$) and the "other taxa" group ($F = 8.93$, $P = 0.0004$).

The Hess sampling was the most efficient technique (MANOVA, Wilk's λ, $F = 7.86$, $P < 0.0001$) with an average of 86% of total individuals obtained in the first sample. The Surber technique was similar in efficiency to the Hess but was not significantly different from the area-restricted electrobug technique ($P > 0.05$). The Hess sampler was more efficient for most groups (Fig. 3). The probability of capture for an invertebrate on a single pass (or efficiency) for the depletion sampling technique was 73% on average. However, 92.5 ± 6.1% (mean ± 1 SE) of the invertebrates were removed after three passes with the electroshocker, determined from Hess samples taken after shocking. Surprisingly, efficiency estimates for the depletion were low for the "other taxa" group but only significantly different from those of the Hess. The

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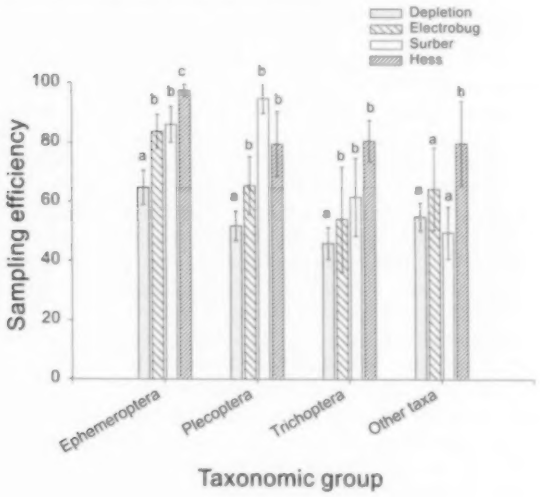
Fig. 3. Sampling efficiency (mean ± 1 SE) for the electroshocking depletion, area-restricted electrobug, Surber, and Hess sampling techniques. Means with different letters are significantly different (adjusted least squares means, $P < 0.05$). Data are from one stream sampled in 1999.



Levene (1960) test for homogeneity of variances in efficiency among the five sampling methods was not significant for overall efficiency or for the individual groups ($P > 0.10$), indicating that the variability in efficiency among replicate samples was similar for each method.

Size distribution of *Baetis* did not differ between samples collected using the Surber and those collected using the electroshocking technique (Kolmogorov–Smirov asymptope (KSa) = 0.9337, $P = 0.3479$, $n = 75$ for females; KSa = 0.6573, $P = 0.7805$, $n = 45$ for males).

Sample processing time was 40% faster for samples taken with the area-restricted electrobug technique than for Surber samples because electrobug samples contained less sediment and detritus. It took 15 min to pick and identify 100 invertebrates from samples taken using area-restricted electroshocking compared with 25 min for the Surber method (Student's t test: time, $t_{28} = 2.52$, $P = 0.0176$; detritus, $t_{28} = -7.41$, $P < 0.0001$) (Table 1).

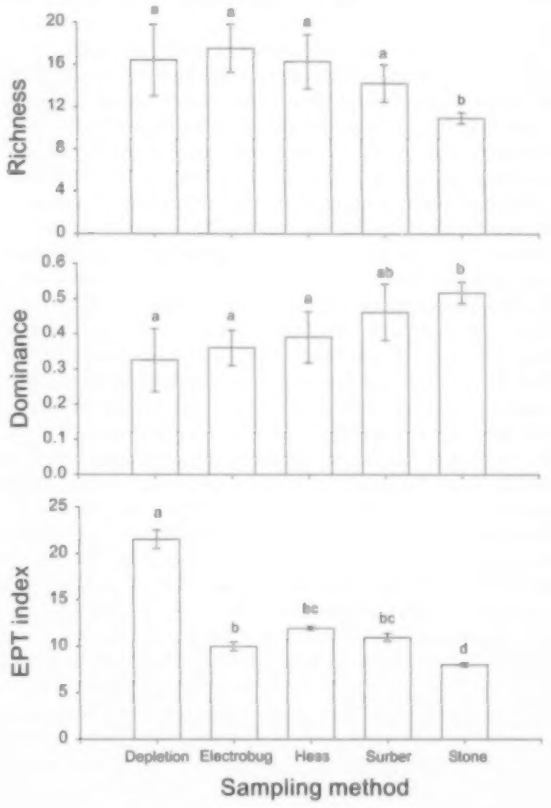
The depletion, area-restricted electrobug, Hess, and Surber methods all had similar estimates of taxa richness, whereas the individual stone sampling method had low taxa richness (Fig. 4). Stone samples were composed mostly of Ephemeroptera taxa, resulting in higher percent dominance scores. The depletion sampling method had significantly higher EPT richness than the other methods; the three area-restricted samplers had similar EPT richness, and individual stone samples had the lowest EPT richness (EPT richness determined by rarefaction). Both electroshocking techniques sampled taxa that commonly attach to rocks (e.g., Blephariceridae larvae).

There were no effects of electroshocking on survival, growth rate, development, or drift behavior of *Baetis* (MANOVA, Wilk's λ, $F = 1.163$, $P = 0.3683$) (Table 2). Retrospective power analyses indicated a low chance of type II error in this analysis for growth rate (power = 94%), mortality (power = 99%), drift (power = 85%), and emergence (power = 61%).

Table 1. Time to process 100 invertebrates and amount of detritus (inorganic and organic material) for the area-restricted electrobug and Surber sampling techniques. Values are means (±1 SE).

Sampling method	Time (min·100 invertebrates^{-1})	Detritus (g·m dry mass^{-2})
Electrobug	15.7 (1.50)	8.79 (1.89)
Surber	25.2 (2.80)	273 (35.7)

Fig. 4. Rarefaction diversity indices (taxa richness, percent dominance of the most common taxon, and EPT richness index) (mean ± 95% confidence interval) for samples taken using the electroshocking depletion, area-restricted electrobug, Hess, Surber, and individual stone sampling techniques. All samples were rarefied to an abundance level of 250.



Discussion

This study provides a new and efficient method for sampling invertebrates that has broad application to stream ecology. Processing benthic invertebrate samples from streams is a laborious task that often constrains observational, experimental, and biomonitoring studies. Investigators are frequently forced to take fewer than the optimal number of

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Table 2. Mean (±1 SE) percent mortality, growth rate, number drifting, and percent emerged for *Baetis* collected using electroshocking and kick sampling.

Response variable	Electroshocking	Kick sampling
Percent mortality	2.1 (1.10)	1.00 (0.07)
Growth rate ($mg \cdot day^{-1}$)	0.1 (0.02)	0.06 (0.02)
Number drifting (min^{-1})	3.0 (0.44)	2.90 (0.50)
Percent emerged	5.4 (1.64)	4.00 (1.75)

samples because of financial constraints and (or) because of the long delay involved in sample processing. The new methods that we present provide estimates of invertebrate density, species richness, and biotic indices comparable with those of traditional methods and take significantly less processing time and cause less disturbance to the stream bottom. We have also used this method to save precious field time when collecting large numbers of live specimens for experiments (Peckarsky and McIntosh 1998; McIntosh and Peckarsky 1999). In addition, it has enabled us to collect and count samples of live invertebrates for density estimates in the field, alleviating the delay between field sampling and laboratory processing.

Without disturbing the stream bottom, the electroshocking techniques provided estimates of the invertebrate density comparable with those of traditional techniques. The Surber sampler, which is partly enclosed on the sides but open on the upstream end, consistently underestimated densities of mobile taxa such as mayflies, which are strong swimmers (e.g., *Baetis*). When Surber samplers are placed in fast-flowing streams, the fine nets may create enough resistance to form eddies or back-flow, which may enable highly mobile taxa to escape. We suspect that the differences in density estimates of the depletion method compared with the various other methods reflected actual differences in habitat sampled because the depletion technique integrated over a broad array of habitats. For instance, depletion sampling would effectively sample invertebrates in bryophytes along the undercut streambank that are not easily sampled by the quadrant or stone techniques. Also, the depletion method was the most effective in capturing riffle-beetle larvae (Elmidae) because it sampled woody debris where they are commonly found. The probability of being captured in one sampling bout (efficiency) was similar among various methods. However, efficiency for stonefly taxa using the electroshocking techniques was low (65%) because stoneflies seldom enter the drift unless the substrate is physically disturbed. On the other hand, Ephemeroptera taxa such as *Baetis* were easily induced to drift and had a high probability of capture in the first pass (depletion: $P = 92\%$; electrobug: efficiency = 96%). The low efficiency for the "other taxa" group was the result of a poor depletion curve for Oligochaeta and Chironomidae, which did not exhibit a sharp decline after only three passes. Estimating population size using removal methods is only appropriate if a large proportion of the population can be collected (Zippin 1956; Cowx 1983). The depletion method removed >90% of the invertebrates after three passes with the electroshocker. Similarly, Frost et al. (1971) found that disturbing the substrate two times reduced total invertebrate density by 60% and an additional disturbance decreased density 30% more. Despite the low density estimates for the Surber sampler, it had a high apparent efficiency, which we suspect is an artifact of highly mobile taxa leaving the sample area and thus not being captured in the second sample.

An alternative method to area-restricted samplers that has become increasingly used is sampling individual stones (Peckarsky 1991; Scrimgeour et al. 1993), which can reduce processing time because less area of stream bottom is sampled, resulting in fewer invertebrates to sort and identify. However, this method is biased toward taxa associated with large stones and underestimates those associated with interstitial spaces, fine sediments, leaf packs, or woody debris. Moreover, to express estimates of invertebrate density on a per square metre scale by this method requires knowing the availability of comparable substratum and the relationship between different substrate sizes and invertebrate density. Simply extrapolating from an individual stone to 1 m^2 of stream bottom assumes that the stream is homogeneous, which is seldom true, and may result in overestimates of population size. Thus, individual stone sampling will overestimate total invertebrate density and underestimate species richness. Based on comparisons of the five sampling techniques that were rarefied to standardize for number of individuals (Sanders 1968; Gotelli and Entsminger 1999), as expected, the individual stone samples had the lowest taxa and EPT richness and the highest dominance. Thus, individual stone sampling was taxon specific, relevant at only small scales, and resulted in population overestimates when extrapolated to larger scales.

Taxa and EPT richness

Knowledge of the number of different taxa in a community is often central to both basic and applied studies. Typically, multiple samples are taken from a community to estimate richness, and as more samples are taken and more individuals are included, richness increases to an asymptote indicating the maximum richness. This phenomenon, or collector's curve (Colwell and Coddington 1994), also applies to comparisons of different sampling techniques, in that each technique may collect different numbers of invertebrates due to the effectiveness of the technique as well as the area sampled. A larger sampling area usually includes more types of habitats and thus more species (Arrhenius 1921; Slocomb and Dickson 1978). However, it is impossible to simultaneously control for sample area and invertebrate abundance (James and Wamer 1982). In this study, differences in sample area are viewed as an attribute of each sampling method. The use of rarefaction was especially important in this study because it enabled accurate comparisons of richness for different sampling methods independent of abundance. Differences in abundance can also be problematic in applied or basic research when comparisons among sites or treatments differ in number of individuals (Vinson and Hawkins 1996; McCabe and Gotelli 2000).

The high taxa and EPT richness for the depletion sampling technique could be attributed to sampling a larger area and thus more habitat types, or the two additional passes may have increased the capture of rare taxa (Pusey et al. 1998). Whatever the mechanism, this method yielded relatively high richness values and is a useful procedure for

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obtaining an accurate measure of the taxa richness of a community.

Electroshocking may be taxon and size specific. We examined the body size distributions of one mayfly taxon, *B. bicaudatus*, and found no evidence for size selectivity with the electroshocking apparatus used and under the environmental conditions of the sampling sites. Electricity has been shown to be effective for inducing mayflies to drift (Elliot and Bagenal 1972; Brown et al. 2000). Within the range of streams in this study, the environmental conditions (e.g., conductivity), and the electroshocking apparatus used, there was little evidence that the electroshocking technique was taxon specific. However, other studies that have investigated the effects of electricity on invertebrates (Bisson 1976; Mesick and Tash 1980; Brown et al. 2000) indicate that the effectiveness can vary according to environmental conditions and electroshocking apparatus.

Advantages of electrobug techniques

Physically disturbing the substrate to sample invertebrates has been the universal standard, but these methods have three major problems. First, large amounts of inorganic and organic debris are included in these samples, which makes sorting difficult and often requires staining to assist in separating organisms from debris. Subsampling has been used to decrease sample processing time but can reduce the accuracy of measurements and loss of rare taxa (Barbour and Gerritsen 1996; Courtemanch 1996; Vinson and Hawkins 1996). Second, physical disturbance of the substratum can slow the recolonization of areas sampled. Traditional techniques are also destructive of resources (algae or organic matter). Third, it is only practical to sample a small area by disturbing the substrate.

The electrobug technique provides solutions for all of these problems. Foremost, the use of electroshocking enables comparable estimates of the invertebrate community to be made with no physical disturbance to the stream bottom. Less detritus in samples makes extracting invertebrates much easier. In addition, repeated sampling of a site is possible without long-term physical disturbance. Furthermore, the electrical current produced from the electroshocking unit is unlikely to affect the algal community or detrital particles (Pringle and Blake 1994; Brown et al. 2000).

Because invertebrate distributions are highly aggregated, devices that sample a small area relative to what is available require more samples to increase the probability of sampling each aggregation and thus decrease the variance (Needham and Usinger 1956; Allan 1982; Morin 1985). The need for high replication to reduce sample variance below 20% was discussed by Allan (1982) and was proposed as one possible problem in detecting differences among locations. The electroshocking depletion technique samples a large area, integrating many habitats and aggregations into one sample. Large numbers of small sampling units have been considered more cost-effective and precise than a few large-area samples (Pringle 1984). However, large-area sampling (e.g., depletion) may be more precise if the mean density (which influences variability) and the number of samples required to provide acceptable variance are known in advance. Morin (1985) has shown that the number of samples necessary to obtain an average standard error of 20% of the mean decreases with increasing sample size and invertebrate density.

Caveats, improvements, and opportunities for innovation

Using electricity for sampling may have negative effects on nontarget organisms such as amphibians but not invertebrates (also see Mesick and Tash 1980). The electroshocking technique requires hauling an electrofishing machine around and two people to sample. Although the cost of an electroshocking machine is not trivial, it may be offset by financial savings in processing time, the increase in turnaround time of samples, and its application for other purposes (i.e., electrofishing). The large-area depletion method will not be practical for large rivers, but in small streams, it may be the most accurate method for detection of differences in the mean abundance of invertebrates when large-scale comparisons are made.

The application of this method may be limited if stream physical characteristics differ from those of Rocky Mountain streams. In terms of physical characteristics, the electroshocking technique, like many stream sampling methods, relies on water velocity to carry invertebrates dislodged from the substrate into a collection net held downstream. As a result, electroshocking may not work well in low-gradient, slow-flowing streams. The type of substrate, be it rocky cobble bottom, fine sediment, or bedrock, will probably have little influence on the effectiveness of the electroshocking method, provided taxa respond and the substrate is not conductive.

We have obviously only tested our method in Rocky Mountain streams where the dominant taxa were mayflies (Ephemeroptera). At our field site, chironomids are not abundant (comprising ~30% of the "other taxa" group). Interestingly, we did capture many invertebrates that we did not expect to, including free-living caddisflies (Rhyacophilidae), net-spinning caddisflies (Hydropsychidae), net-winged midges (Blephariceridae), and chironomids (Chironomidae). This method will not work well for collecting invertebrates with heavy cases or shells, such as snails or stone-cased caddisflies, because they do not drift easily.

The electroshocking techniques have broad application for sampling, collecting, and manipulating invertebrates for experiments. Electrical devices have also been developed to exclude freshwater shrimp (Pringle and Blake 1994) or sample marine arthropods (Phillips and Scolaro 1980). Studies in New Zealand, Venezuelan, and North American streams have used an electroshocker and a collection net held downstream to collect large numbers of invertebrates (Penczak and Rodriguez 1990; McIntosh and Townsend 1994; Fièvet et al. 1996) for surveys of species richness (Rabeni et al. 1997) or for experiments in which large numbers of invertebrates were needed (Peckarsky and McIntosh 1998; McIntosh and Peckarsky 1999). Although the electroshocking technique may not work well for all taxa or in every stream type, modifications can be made to apply this technique for a variety uses and across many systems. For example, the depletion electrobug method may enable sampling of habitats that are not accessible using traditional methods (e.g., bedrock streams or debris dams). Furthermore, our ability to remove ~90% of the invertebrates from a

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large area of stream may provide opportunities for innovations involving large-scale manipulations of invertebrates in natural streams using electricity (B.W. Taylor, unpublished data).

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Examining feeding strategies and position choice of drift-feeding salmonids using an individual-based, mechanistic foraging model

G.R. Guensch, T.B. Hardy, and R.C. Addley

Abstract: We demonstrated the ability of a mechanistic habitat selection model to predict habitat selection of brown trout (*Salmo trutta*) and mountain whitefish (*Prosopium williamsoni*) during summer and winter conditions in the Blacksmith Fork River, Utah. By subtracting energy costs and losses from the gross energy intake rate (GEI) obtained through simulation of prey capture, the model calculates the potential net energy intake rate (NEI) of a given stream position, which is essentially the rate of energy intake available for growth and reproduction. The prey capture model incorporates the size, swimming speed, and reaction distance of the fish; the velocity, depth, temperature, and turbidity of the water; and the density and size composition of the drifting invertebrates. The results suggest that during both summer and winter, the brown trout and mountain whitefish in our study reach avoided locations providing low NEI and preferred locations providing a high ratio of NEI to the swimming cost (SC) at the focal position of the fish (NEI/SC). This supports the idea that the drift-feeding fish in this study selected stream positions that provided adequate NEI for the least amount of swimming effort.

Résumé : Un modèle mécaniste de sélection d'habitat a pu prédire efficacement les choix d'habitat de la Truite brune (*Salmo trutta*) et du Némomini des montagnes (*Prosopium williamsoni*), tant en été qu'en hiver, dans la rivière Blacksmith Fork en Utah. En soustrayant les coûts et les pertes énergétiques du taux brut d'ingestion d'énergie (GEI) obtenu par simulation de captures de proies, le modèle calcule le taux potentiel net d'ingestion d'énergie (NEI) pour une position donnée dans le cours d'eau, ce qui correspond essentiellement aux taux d'ingestion d'énergie disponible pour la croissance et la reproduction. Le modèle de capture des proies tient compte de la taille du poisson, de sa vitesse de nage et de sa distance de réaction, de la vitesse du courant, de la profondeur, de la température et de la turbidité de l'eau, ainsi que de la composition en fonction de la taille et de la densité des invertébrés dans la dérive. Les résultats indiquent qu'en été aussi bien qu'en hiver la Truite brune et le Némomini des montagnes évitent les régions du cours d'eau qui fournissent des NEI bas et préfèrent les zones qui permettent d'obtenir un NEI élevé par rapport au coût de la nage (SC) à la position focale du poisson (NEI/SC). Ces résultats appuient l'hypothèse qui veut que les poissons de notre étude, qui s'alimentent dans la dérive, choisissent dans le cours d'eau les positions qui leur fournissent des taux suffisants de NEI pour le moindre effort de nage.

[Traduit par la Rédaction]

Introduction

The scientists, biologists, and engineers charged with managing aquatic resources often rely on habitat models to estimate the outcome of flow, morphological, and watershed alterations. The physical habitat simulation system (PHABSIM), in particular, is a commonly used set of simulation tools for this purpose (Reiser et al. 1989). This type of approach is sufficient when (*i*) the appropriate life stages of the target species have adequately defined habitat preferences; (*ii*) the bathymetric, hydraulic, and hydrologic characteristics of the system are accurately measured or simulated; and (*iii*) physical habitat quantity and (or) quality is indeed the limiting agent (Hardy 1998). Some criticize PHABSIM, however, because it relies on empirically based suitability curves that lack specific biological mechanisms (Orth and Maughan 1982).

Numerous researchers have suggested that a bioenergetic approach will better link habitat models and biological mechanisms (e.g., Hughes 1992; Hayes 1996; Van Winkle et al. 1998). Some advantages of a bioenergetic approach are that it provides information on mechanisms underlying observed phenomena, it is inherently linked to growth and biomass production, it is theoretically more transferable among systems, and it is connected intrinsically to the food web dynamics of the system. In addition, continued advancements in computing power and the potential for spatial data collection will make such spatially explicit modeling efforts more widely feasible (Hardy 1998).

A bioenergetic approach also has the potential to estimate the growth and production of an ecological system. In lotic environments, however, the feeding conditions vary substantially with the location of the fish. As a result, habitat selec-

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tion must be predicted or provided as an input before bioenergetic models can estimate growth potential in these systems. Numerous authors have attempted to use bioenergetic models to predict habitat selection (e.g., Hughes 1992; Hill and Grossman 1993; Hayes 1996) or habitat suitability (Braaten et al. 1997; Baker and Coon 1997) with varying degrees of success.

Addley (1993) originally developed the model used herein for cutthroat trout (*Oncorhynchus clarki*) in St. Charles Creek, Utah. The model, which combines the Holling (1959) disk equation for predation and concepts from the prey capture model of Hughes and Dill (1990), calculates the potential net energy intake rate (NEI) of a given stream position by subtracting energy costs and losses from the gross energy intake rate (GEI) obtained through simulation of prey capture. Since all energy costs and losses are accounted for, NEI is essentially the intake rate of energy that will be available for growth and reproduction. Addley (1993) also tested the model and performed a sensitivity analysis. He concluded that all except the smallest size-class of Bear Lake cutthroat trout (*Oncorhynchus clarki utah*) selected habitats providing high NEI in St. Charles Creek and that the model was most sensitive to swimming velocity, reaction distance, and drift characteristics. Ludlow (1998) also tested the NEI model of Addley (1993) and demonstrated that the brown trout (*Salmo trutta*), mountain whitefish (*Prosopium williamsoni*), and rainbow trout (*Oncorhynchus mykiss*) observed in the Logan River, Utah, used high-NEI stream positions and avoided positions with negative or low NEI values.

The objective of this study was to evaluate the ability of the Addley (1993) bioenergetic model to predict habitat utilization in a river with physical characteristics different from either of the rivers where the model was previously tested. Specifically, our study site on the Blacksmith Fork River in northeastern Utah had a lower gradient, smaller substrates, a more uniform velocity distribution, and less visibility than St. Charles Creek or the Logan River.

General model description

The model evaluated in this study, derived by Addley (1993), simulates NEI by subtracting energy losses from the GEI, which is the estimated total prey energy per hour that a fish can consume in a given feeding location. Energy losses consist of metabolic costs, digestive costs, and nonassimilated energy losses. Metabolic costs include routine basal metabolism while maintaining a station in the current and prey capture costs. Digestive cost is the energy required for prey digestion. Nonassimilated energy is energy egested in feces and excreted as ammonia and urea in urine. Theoretically, NEI is

$$(1) \qquad \mathrm{NEI} = \frac{\text{NET ENERGY GAINED(J)}}{\text{TOTAL TIME(s)}} = \frac{E}{T_s + T_f}$$

where NEI is the rate of energy intake available for growth and reproduction once costs and losses are accounted for, T_s is the time spent searching (seconds), and T_f is the time spent handling prey (seconds). For a drift-feeding salmonid

$$(2) \qquad E = \sum_{i=1}^{n} (\mathrm{DD}_i \mathrm{MCA}_i V_{\mathrm{ave}_i} \mathrm{PC}_i (E_i - \mathrm{CC}_i) T_s) - \mathrm{SC} T_s$$

where DD_i is the drift density of prey size-class i (prey per cubic metre), MCA_i is the maximum capture area (square metres) for prey size-class i (Fig. 1) (to be discussed further in the next section), V_{ave_i} is the mean velocity (metres per second) passing through the MCA, PC_i is the probability of successful capture, E_i is the energy available per prey item (joules per prey) after digestive costs and assimilation losses are subtracted, CC_i is the prey capture cost (joules per prey), and SC is the stationary swimming and basal metabolic cost (joules per hour).

Similarly:

$$(3) \qquad T_f = \sum_{i=1}^{n} \mathrm{DD}_i \mathrm{MCA}_i V_{\mathrm{ave}_i} T_s t_{f_i}$$

where t_{f_i} is the handling time per prey item (seconds per prey).

Substituting the above expressions into the original equation gives

$$(4) \qquad \mathrm{NEI} = \frac{\sum_{i=1}^{n} (\mathrm{DD}_i \mathrm{MCA}_i V_{\mathrm{ave}_i} \mathrm{PC}_i (E_i - \mathrm{CC}_i) T_s) - \mathrm{SC} T_s}{T_s + \sum_{i=1}^{n} \mathrm{DD}_i \mathrm{MCA}_i V_{\mathrm{ave}_i} T_s t_{f_i}}.$$

Canceling T_s then results in our NEI equation, which agrees with that of Charnov 1976:

$$(5) \qquad \mathrm{NEI} = \frac{\sum_{i=1}^{n} (\mathrm{DD}_i \mathrm{MCA}_i V_{\mathrm{ave}_i} \mathrm{PC}_i (E_i - \mathrm{CC}_i)) - \mathrm{SC}}{1 + \sum_{i=1}^{n} \mathrm{DD}_i \mathrm{MCA}_i V_{\mathrm{ave}_i} t_{f_i}}.$$

Equation 5 is essentially a formulation of the Holling (1959) disk equation that incorporates multiple prey classes and the swimming cost (SC) associated with holding a search position in the current. Only the basic equations are included in the text. Table 1 provides a complete summary of the model equations.

The calculation of MCA assumes that the fish maintains a holding position in the current and makes forage attempts for drifting prey (Fig. 1). The MCA is roughly a partial circle or partial ellipse shaped area that is perpendicular to the fish's orientation (i.e., perpendicular to stream flow) within which the fish can capture prey before it drifts past. The shape and size of the MCA are determined by the maximum capture distance (MCD, metres), which are calculated in radial directions out from the focal position based on the combination of reaction distance, water velocity, and the potential swimming speed used by the fish during forage attempts. The 60-min maximum sustainable velocity ($V_{\max}$, metres per second) is used to estimate the potential swimming speed of foraging fish.

The MCD is computed in all radial directions from the focal point of the fish in a plane perpendicular and transverse to the fish and the current (Fig. 1). The MCD is generally

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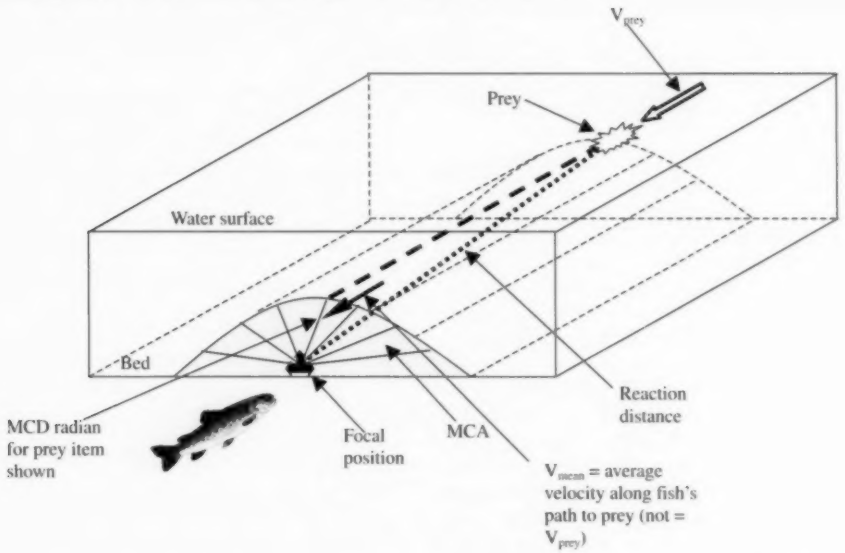
Fig. 1. Diagram showing geometric variables in mean capture area (MCA) calculation.



different in each radial direction. It can be smaller vertically than laterally, for example, because of increased water velocity higher in the water column. Specifically, the MCD is determined by setting the time required for a prey item to pass the fish after it is spotted (reaction distance upstream) equal to the time required for the fish to intercept the prey. This process results in the following equation for MCD:

$$\text{(6)} \qquad \text{MCD}_i = \sqrt{\frac{\text{RD}_i^2(V_{\max}^2 - V_{\text{mean}_i}^2)}{V_{\text{prey}_i}^2 + V_{\max}^2 - V_{\text{mean}_i}^2}}$$

where RD is the reaction distance of the fish (centimetres) to the ith prey size, V_{prey_i} is the velocity of the prey (metres per second), and V_{mean_i} is the mean water velocity (metres per second) along the ith MCD radial. This equation is solved with an iterative computer program because V_{mean} is a function of MCD.

RD is a function of fish size, prey size, light levels, and turbidity (Dunbrack and Dill 1983; Henderson and Northcote 1985; Barrett et al. 1992). Addley (1993) derived the equation for RD shown in Table 1 from the empirical data of Dunbrack and Dill (1983). Table 2 shows the prey size distribution and the resulting RD estimates.

The MCA is then calculated as the sum of the incremental areas associated with each MCD radian perpendicular to the flow and the fish (Fig. 1):

$$\text{(7)} \qquad \text{MCA}_i = \sum_{j=1}^{m} \frac{d\theta}{2} \text{MCD}_{ij}^2 .$$

In eq. 7, $d\theta$ is an incremental angle perpendicular to flow vectors and the fish that is associated with each MCD radial. We used $m = 10$ to provide a half-ellipse shaped capture window with θ ranging from 0.0 to 3.14 radians and $d\theta = 0.314$.

The input values used in the model runs were the following: fish size (millimetres), temperature (summer, 12°C; winter, 6.2°C), minimum depth (15.25 cm), focal depth (brown trout, 15 cm; mountain whitefish, 3 cm), percent trout $V_{\max}$ (brown trout, 1.0; mountain whitefish, 0.8), turbidity (12 NTU), total drift density (summer, 7.8 prey·m^{-3}; winter, 14.1 prey·m^{-3}), and incremental distance (3.05 cm). Of those input values, fish size, temperature, turbidity, and drift density were measured, while minimum depth, focal depth, and percent trout $V_{\max}$ were estimated from observation. The velocities throughout the water column were determined from the mean velocities using the following empirical velocity equation from Milhouse (1990):

$$\text{(8)} \qquad \frac{V_p}{V} = A\left(\frac{D_p}{D}\right)^B$$

where V is the mean velocity and D is the depth at a given location in the river, V_p is the velocity at depth D_p in the water column, and A and B are empirical constants ($A = 0.919$ and $B = 0.23$).

Model test

Study reach

Our study reach was a 250-m section of the Blacksmith Fork River located on private property in sagebrush-dominated rangeland of northeastern Utah, approximately 10 miles (1.61 km) southeast of Logan, Utah. The elevation of the area is approximately 1750 m, the surficial geology is primarily limestone, and the river at this location is third order. The reach encompassed two meander wavelengths of channel features, which were generally pools, riffles, and runs. Other characteristics of the study reach were a baseflow discharge of 2.4–2.7 m^3·s^{-1}, a gradient of approximately 0.5%, relatively mild spring flooding, high productivity, and

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Table 1. Equations used in the net energy intake (NEI) model (Addley 1993).

Parameter and units	Equation/calculation method	Discussion and citations
NEI_i (J·h^{-1})	$NEI = \frac{\sum_{i=1}^{n} MCA_i V_{ave} DD_i PC_i(E_i - CC_i) - SC}{1 + \sum_{i=1}^{n} t_f MCA_i V_i DD_i}$	Net energy intake rate based on possible gross energy intake minus energy costs and losses for each prey class i
MCD_{ij} (ft)	$MCD_{ij} = \sqrt{\frac{RD_i^2(V_{max}^2 - V_{mean}^2)}{V_{max}^2 + V_p - V_{mean}^2}}$	Maximum capture distance, calculated in the plane transverse and perpendicular to the fish by an iterative computer program, where V_{mean} is the mean velocity along MCD_{ij} (calculated within the computer program) and RD_i is the reaction distance for prey size i
V_{max} (ft·s^{-1})	$V_{max} = 13.86\left(\frac{21.42 - T}{3.92}\right)^{0.24} e^{0.24\left(1-\left(\frac{21.42-T}{3.92}\right)\right)} TL^{0.63}$	Maximum sustained fish velocity equation derived from Brett and Glass (1973), where T is temperature (°C) and TL is total length (cm)
RD_i (ft)	$PL_i = (RD_i^2 + 50RD_i)\left(\frac{1 + 1.58e^{-0.0334(T)}}{1725}\right)$	Reaction distance equation derived from data of Dunbrack and Dill (1983), where PL_i is prey length (mm), RD_i is reaction distance (cm), and T is total fish length (cm)
RD_i' (ft)	$RD_i' = \frac{RD_i(-2.27\,TURB + 100)}{100}$	Reaction distance from equation above adjusted for turbidity (TURB) with equation adapted from Barrett et al. (1992)
V_{mean_j}	Computed within computer program from velocity data	Average velocity along MCD radian j for prey class i
MCA_i (ft^2)	Area circumscribed by the arc created by connecting the ends of the MCD_i radians in the plane transverse and perpendicular to the fish (calculated with a computer program)	Maximum capture area at a location given water depth, water velocity, and channel morphology
V_{ave} (ft·s^{-1})	Computed within computer program from velocity data	Average water velocity in the MCA for prey class i
DD_i (prey·ft^{-3})	Site-specific emperical data	Measured daytime drift density in for each prey size i
PC_i	Assume probability of capture equals 1.0	Probability of successful prey capture
PE_i (J·prey^{-1})	$PE_i = 0.3818(PL_i)^{2.46}$	Prey energy derived from Smock (1980) and Cummins and Wuycheck (1971), where PL_i is prey length (mm).
E_i (J·prey^{-1})	$0.58PE_i$	Energy assimilated (gross energy intake minus 14% for food digestion and 28% for losses due to excretion and feces)
TC_i (s·prey^{-1})	$TC_i = \frac{2MCD_i}{(V_{max}^2 - V_{mean}^2)^{1/2}}$	Swimming time required for fish to capture a prey item 1 MCD away
CC_i (J·prey^{-1})	$CC = \frac{6}{3600} SC(V_{max})TC$	First approximation of prey capture cost: six times cost of steady swimming (SC) for 1 s at V_{max} times the capture time (TC). This estimate falls at the lower end of the range (5–20) found empirically by Puckett and Dill (1984), at the lower end of the range (6–14) derived empirically by Boisclair and Tang (1993), and in the middle of the range (2.6–10) estimated by Hughes and Kelly (1996)
SC (J·h^{-1})	$SC = 1.4905\ W^{0.784} e^{0.0687T} e^{(0.0259 - 0.0005T)V_{cm}}$	Steady swimming cost from Stewart (1980), where W is weight (g), T is temperature (°C), and V is velocity (cm·s^{-1})
t_f (s)	Empirically approximated as 5 s	Estimated total time required for fish to recognize, intercept, attack, and swallow a prey item and then resume searching at the focal position (from Bachman 1984)

Note: 1 ft = 0.31 m.

moderate visibility. Additionally, summer water temperature averaged approximately 10.5°C and ranged daily between 8°C and 13°C, and the winter water temperature averaged approximately 5°C and ranged between 3.5 and 6.5°C. Introduced brown trout and the native mountain whitefish dominated the fish population in the study reach.

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Table 2. Prey size classes and percentages of total drift density and reaction distances and minimum mean capture area (MCA) values used as model input for all size-classes of brown trout (BT) and mountain whitefish (MW).

	Prey class					Minimum MCA (cm^2)
	1	2	3	4	5	
Size (mm)	10	8	6	4	2	
Summer %	0.1	0.9	5.0	43.0	51.0	
Winter %	0.01	0.09	01.9	30.0	68.0	
Reaction distance (cm)						
90-mm BT	93.9	81.7	68.3	52.6	32.6	0.93
165-mm BT	107	93.9	78.9	61.0	38.4	3.7
254-mm BT	109	95.1	79.9	61.9	39.0	5.6
350-mm BT	109	95.1	79.9	61.9	39.0	7.4
280-mm MW	109	95.1	79.9	61.9	39.0	3.7
370-mm MW	109	95.1	79.9	61.9	39.0	4.6
460-mm MW	109	95.1	79.9	61.9	39.0	5.6

Oncorhynchus clarki utah and sculpin (*Cottus*) were also observed on rare occasions. The suspended sediment content of the water was moderate, making the sight distance at which reliable fish observations could be made only 1.5–2.0 m.

Mapping of bathymetry and hydraulics

We developed a 0.914-m grid system to map the physical features of the study reach in an efficient and repeatable manner. For each water stage height observed during data collection, we measured the water surface profile and took a discharge measurement at a fixed cross section by the stage plate. We recorded depth, velocity, flow direction, and substrate at each grid point. We then back-calculated the bed elevation by subtracting the depth from the water surface elevation given by the measured water surface profile. This approach was accurate to within 2 cm in most areas and 4 cm in the small riffles with excessive surface turbulence. In either case, the accuracy was well within the resolution of the 0.914-m grid. In ESRI ArcView version 3.1, we smoothed the depth, velocity, and bed elevation data into 0.3-m grids. Substrate categories were cobble, gravel, sand, silt, vegetation, compacted silt–travertine, and large woody debris.

Observation methods

We used snorkeling and remote observation multiple times per day for 15 days during summer and for 2 days during winter to determine habitat selection by the resident fish populations within the study reach. Moderate visibility, the presence of silts, and the skittish nature of brown trout complicated direct observation by snorkeling. Therefore, we built two 3-m-high towers to allow remote observation. Snorkeling was necessary in sections where the depth exceeded 1 m, surface turbulence limited visibility, or tree cover prevented remote observation.

Underwater observation

In sections where snorkeling was necessary, we employed techniques similar to those of Fausch (1984) and those outlined in Thurow (1994). The snorkeler entered the stream approximately 25 m downstream of the study reach and proceeded upstream slowly using the substrate as much as possible for propulsion. Upon sighting a fish, the snorkeler remained motionless for as long as was necessary to determine if the fish had been disturbed, usually 1–5 min. If the fish was undisturbed, the diver then noted the species, size (centimetres), distance from bottom (centimetres), and the activity state (feeding or hiding/resting) of the fish. The resolution of fish size and distance from bottom estimates was within 2.5 and 3 cm, respectively. This information was then associated with a numbered tag that the diver placed beneath the observed focal point. Fish were considered to be feeding if seen making forage attempts or if seen actively maintaining a focal position in the current. We considered all fish lying motionless under banks, on the bottom, or within plant cover to be hiding or resting and used those observations for population data only.

Remote observations

We recorded remote observations several times a day for approximately 18 days during summer and for 5 days during winter from the observation towers and occasionally from high banks. The duration of remote observations was generally between 15 and 30 min. We recorded positions on scale maps of the study reach, and the same information was noted as in the snorkeling surveys. Eventually, we placed weighted tags in feeding sites identified by remote observations.

Tag measurement

Once the primary feeding sites within the study reach were identified, the observer went through the reach and measured the location of the tags in the grid system. At each tag, we measured a vertical velocity profile and recorded the focal velocity. In addition, we noted the substrate and description of the immediate area. We referenced these data to the tag number and the associated fish observation history and then entered them into a database format and imported into ESRI ArcView version 3.1 for visual display with the physical data and model results.

Macroinvertebrate sampling

We established two drift sampling stations within the study reach: (*i*) four nets 30 m upstream of the large recirculating pool in a deep run and (*ii*) three nets, placed laterally,

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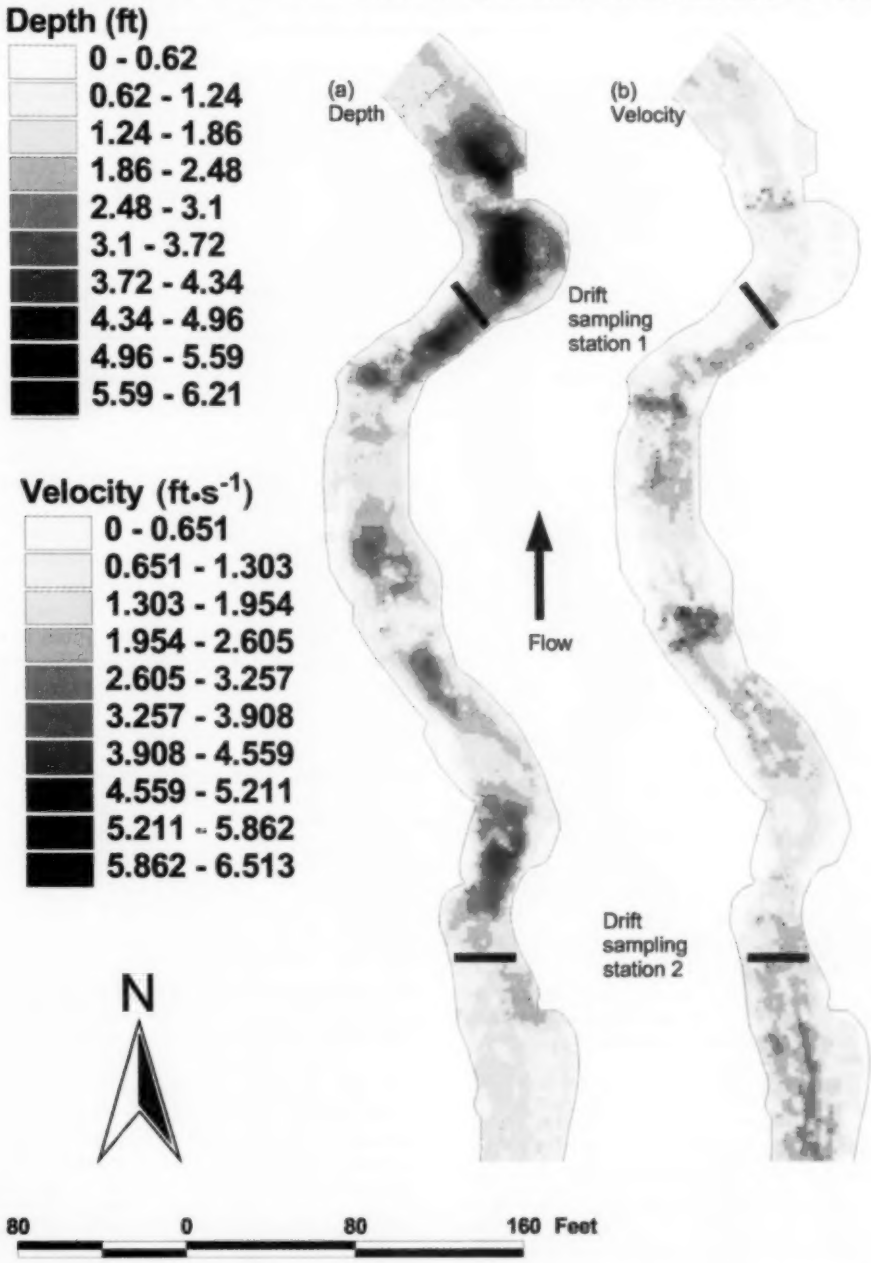
Fig. 2. Contour image of depths and velocities in the study reach showing the locations of drift sampling stations (1 ft = 0.31 m).



at the upstream end of the reach in a shallow run (Fig. 2). We sorted drift samples selectively in order to obtain density and size distribution estimates for model input in light of time and budget constraints. We sorted seven samples of triplicates for the late-summer model run from sampling site 1 (Fig. 2), which was located slightly downstream from the center of the study reach. We collected these samples in early September during the latter half of the fish observations. Three samples were collected at sunrise, two during the afternoon, and two at sunset. We also collected two triplicate samples during February to estimate the drift and size distribution for the winter model runs. We used the same sampling site as the one used for the September drift samples and collected one sample at sunrise and the other during the afternoon.

Data analysis

Because NEI is a function of numerous physiological variables that are functions of fish size, it was necessary to apply the model to discrete size-classes. Therefore, we

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Table 3. Drift density (DD) data showing date, time period (M, morning, A, afternoon, E, evening), size-classes, density, and standard deviation (SD) (each entry shown is the average of a triplicate sample).

		Size-class (mm)						
Date	Time period	1–3	3–5	5–7	7–9	9–11	Total DD (prey·m^{-3})	SD (prey·m^{-3})
Sept. 11, 1997	M	6.267	4.651	0.517	0.123	0.000	11.549	4.919
	M	3.439	2.849	0.336	0.026	0.000	6.644	0.929
	M	4.131	2.610	0.308	0.023	0.000	7.066	0.930
	A	5.647	4.157	0.296	0.028	0.000	10.120	1.481
	A	6.212	4.998	0.663	0.159	0.031	12.054	3.781
	E	2.151	2.257	0.410	0.047	0.021	4.883	0.401
	E	2.496	3.562	0.390	0.086	0.000	6.529	1.500
Feb. 12, 1998	M	8.754	3.570	0.257	0.024	0.000	12.595	1.775
Feb. 26, 1998	A	10.970	5.037	0.285	0.000	0.006	16.286	0.891

divided the brown trout and mountain whitefish into four and three respective size-classes based on the size distribution of fish observed during summer: 90 mm (50–125 mm), 165 mm (125–200 mm), 254 mm (200–300 mm), and 350 mm (300–380 mm) for brown trout and 280 mm (200–330 mm), 370 mm (330–406 mm), and 460 mm (406–480 mm) for mountain whitefish. We ran the model specifically for each species and size-class.

We used ESRI ArcView version 3.1 to create color-contour images of the model output grids (NEI and NEI/SC) and the fish tag data. This provided easy visual assessment of the spatial correlation between the NEI and NEI/SC distributions and the observed feeding locations. To determine the NEI and NEI/SC values of the stream locations where fish were observed, we used ESRI ARC/INFO to associate the NEI and NEI/SC values with the appropriate fish tag in the observation database. Using these NEI observed and NEI available (model output) data, we computed a Manly preference index (α) and the associated standard error (Manly 1974) for NEI and NEI/SC.

We also calculated a weighted average of the Manly α distribution (α_{wave}) by multiplying the α for each NEI or NEI/SC class by the upper percentage of that class and summing them. For example, $\alpha_{wave} = \alpha_{80-100} \times 100 + \alpha_{60-80} \times 80 + \alpha_{40-60} \times 60 + \alpha_{20-40} \times 40 + \alpha_{0-20} \times 20$. We approximated the standard error of α_{wave} by summing and weighting the variances and covariances of the α for each class.

Results

Channel bathymetry and hydraulics

The intense sampling regime employed for the depth and velocity measurements provided a data set from which accurate depth and velocity maps could be generated in ESRI ArcView version 3.1 (Fig. 2). These images depict the characteristics of the study reach.

Drift

As shown in Table 3, the majority of the invertebrates were between 1 and 5 mm in length. Generally, the afternoon drift densities were the highest, the morning and evening drift densities were similar, and no measurable vertical or lateral variation was detected. Because fish observations were done at times ranging from early morning to dusk and the diurnal variation of drift density was not excessive, we used the average of the September drift densities (7.8 prey·m^{-3}, SD = 321) as model input for summer. The orders Ephemoptera and Diptera dominated the macroinvertebrate distribution.

For the winter samples, the drift densities were higher in the afternoon than in the morning and generally exceeded summer estimates. Again, because fish were observed at times ranging from early morning to dusk and the diurnal variation of drift density was not excessive, we used the average of the February drift densities (14.1 prey·m^{-3}, SD = 238) as model input for winter. The majority of the invertebrates was again between 1 and 5 mm in length and dominated by ephemopterans and dipterans; however, the winter size distribution was skewed more toward the smallest size-class.

Model output and habitat selection

Summer

The NEI values within the study reach were high in all river locations except the riffles and stream margins (Fig. 3*a*). The pattern of NEI/SC was more heterogeneous than that of NEI, and the high-NEI/SC locations correlated very well with slower, deeper pool habitats and subsequently with observed fish positions (Figs. 3*b* and 3*c*). This pattern is expected because NEI/SC is substantially reduced in high-velocity locations because SC increases exponentially with velocity. The preference index analysis showed that brown trout and mountain whitefish preferred stream locations providing NEI in the upper 60% of the NEI range (Fig. 4*a*). Within this range, however, no clear pattern was evident. For NEI/SC, however, all sizes of brown trout and mountain whitefish showed a high preference for stream locations in the upper 20% of the NEI/SC range (Fig. 4*b*).

Winter

For the winter runs, the NEI and NEI/SC distributions were similar to those of summer. Brown trout in the 165- and 350-mm size-classes showed a preference for stream locations providing NEI in the upper 20% of the NEI range (Fig. 5*a*), although there were only six fish in each of these classes. The 254-mm size-class of brown trout and the 370-mm size-class of mountain whitefish both preferred habitats with above 60% of maximum NEI (Fig. 5*a*). Finally, the 280-mm size-class of mountain whitefish showed the highest preference (α = 0.55) for locations with 20–40% of maxi-

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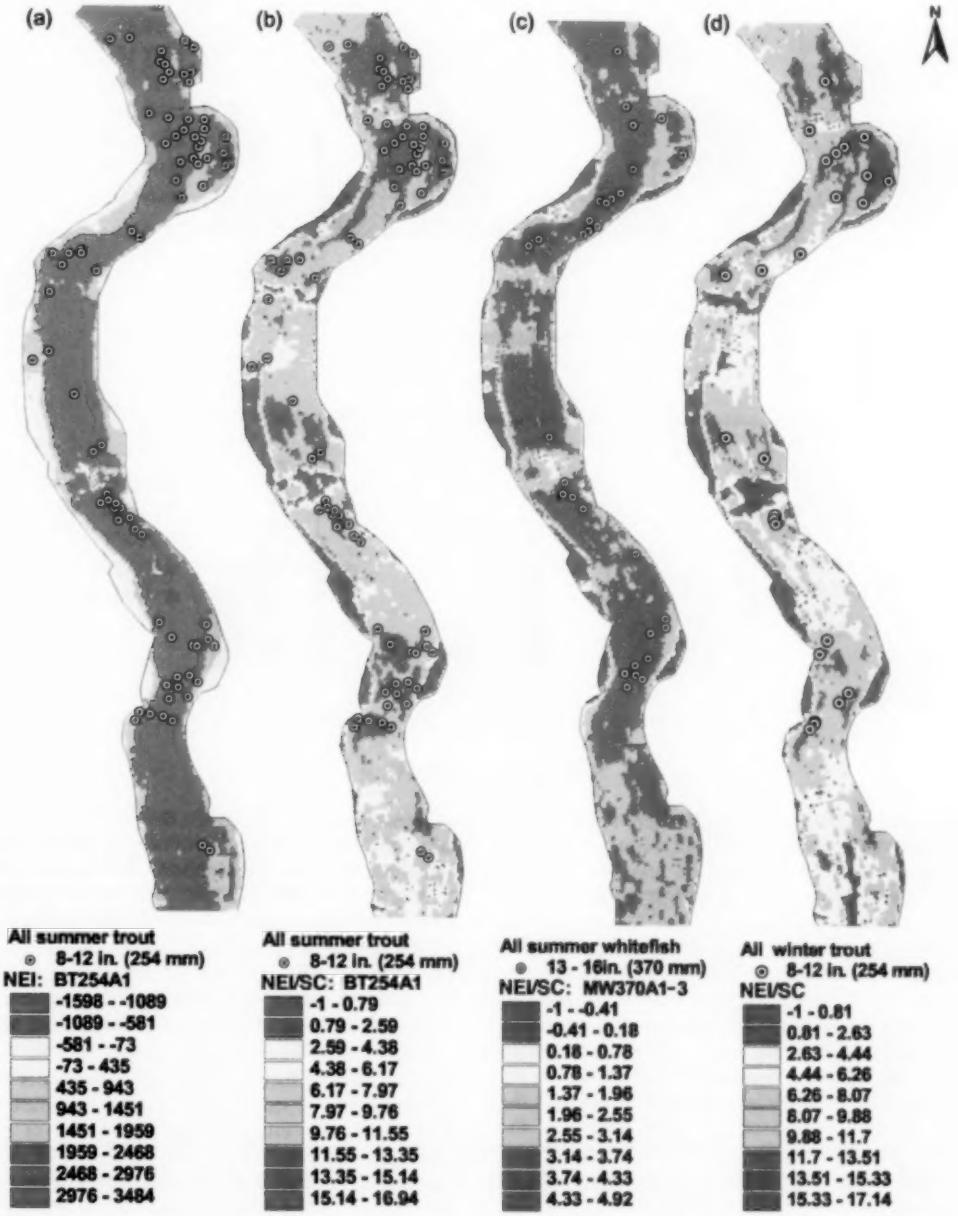
Fig. 3. Spatial distributions of summer net energy intake (NEI, $J \cdot h^{-1}$) and the dimensionless ratio of NEI to the swimming cost (SC) at the focal position (NEI/SC) for the (*a* and *b*) 254-mm size-class of brown trout (BT) and (*c*) 370-mm size-class of mountain whitefish (MW) and (*d*) winter NEI/SC for the 254-mm size-class of brown trout.



mum available NEI and some preference ($\alpha = 0.4$) for locations with 80–100% of the maximum NEI (Fig. 5*a*).

The spatial pattern of NEI/SC was again more heterogeneous than that of NEI, and the high-NEI/SC locations correlated very well with slower, deeper pool habitats and subsequently with observed fish positions (Fig. 3*d*). The 165- and 350-mm size-classes of brown trout, each with a sample size of 6, showed the highest preference for the 40–60% of maximum NEI/SC class (Fig. 5*b*). The 254-mm size-class of brown trout and the 280- and 370-mm size-classes of mountain whitefish, each with more sufficient sample sizes, showed increasing preference for high-NEI/SC stream locations (Fig. 5*b*). The optimal result occurred for the 370-mm size-class of mountain whitefish, where all fish were found in stream locations in the upper 20% of the NEI/SC range (Fig. 5*b*).

Quantitative summary

Weighted averages of Manly's α (α_{wave}) provided a more quantitative metric of model performance that included an

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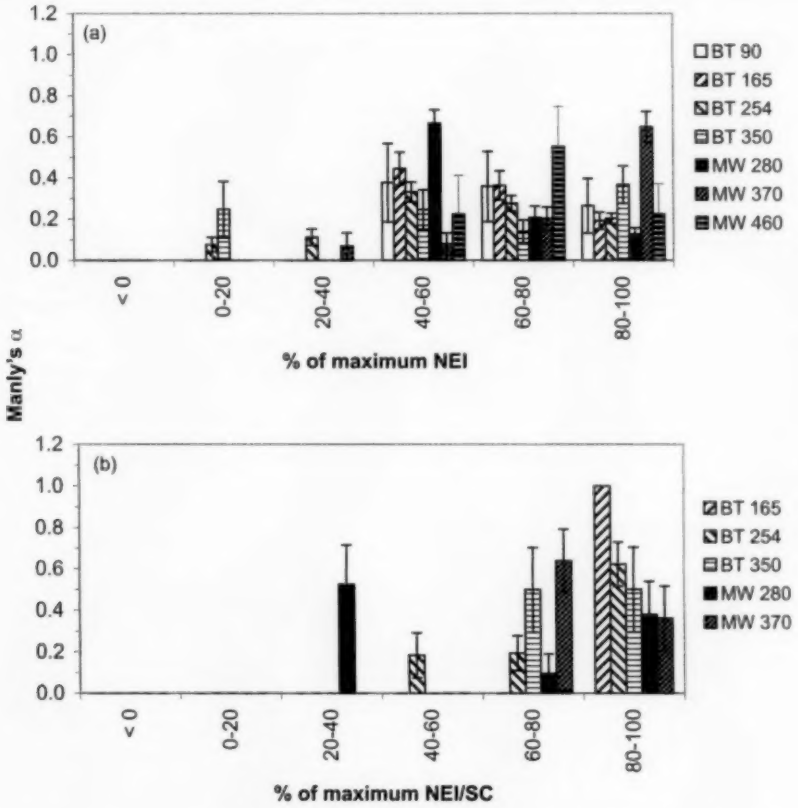
Fig. 4. Manly's α for net energy intake (NEI) computed for all brown trout (BT) and mountain whitefish (MW) size-classes during (*a*) summer and (*b*) winter.



estimate of standard error and the sample size. For summer, the α_{wave} for NEI of all size-classes of brown trout and mountain whitefish were all between 68 and 88, which is high (Table 4). The α_{wave} values for NEI/SC were between 90 and 100 for all fish size-classes except 350-mm brown trout, which was still relatively high at 76 (Table 4). Two fish in positions with LWD and low NEI/SC (the outlier preference for the 20–40% NEI/SC class in Fig. 4*b*) reduced α_{wave} for the 350-mm size-class. All fish size-classes scored high α_{wave} values for NEI during winter with the exception of the 280-mm size-class of mountain whitefish (Table 4). For NEI/SC, the 254-mm size-class of brown trout and the 280- and 370-mm size-classes of mountain whitefish, the three with large sample sizes, had high α_{wave} values, with the latter showing the optimal result (α_{wave} = 100) where all fish were in locations in the highest NEI/SC class (Table 4).

Discussion

The data show that for all fish size-classes with adequate sample sizes, fish avoided locations with negative or very low computed NEI values and preferred locations with high computed NEI/SC values. In our study section of the Blacksmith Fork River, the velocity distribution was relatively uniform, and in nearly all nonriffle locations, the combinations of water velocity and depth were in a range that provided a moderate to high NEI, given the drift density available. The ratio NEI/SC, however, provided a much more heterogeneous pattern that corresponded well to fish positions. The NEI at a given location may provide an estimate of energy intake rate, the accuracy of which was addressed in Guensch (1999) and will be discussed in a subsequent publication. The ratio NEI/SC, however, provides a means to better predict position choice by differentiating between stream locations with different characteristics but high or adequate NEI.

Based on the correlation between NEI/SC and the locations of feeding fish in this study, we suspect that the feeding behavior of salmonids is more complex than simple maximization of either energy intake rate or energy intake efficiency. We propose that the time scale of the rate is very important and that in many cases, fish primarily maximize their energy intake on a long-term or daily basis and secondarily minimize the amount of effort required. If this is the case, then in river systems without strong predation threats and with enough available food for fish to achieve maximum daily consumption, measures of benefit versus effort such as NEI/SC, where SC is a surrogate of the effort expended, should be strongly correlated with fish habitat use, as shown here. Such a scenario corresponds to the limited energy assimilation case discussed in Ydenberg et al. (1992) or a situation where a constraint on total assimilation exists, as discussed in Ydenberg and Hurd (1997). Alternatively, in

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Fig. 5. Manly's α for the ratio of net energy intake to the swimming cost at the focal position (NEI/SC) computed for all brown trout (BT) and mountain whitefish (MW) size-classes during (*a*) summer and (*b*) winter.

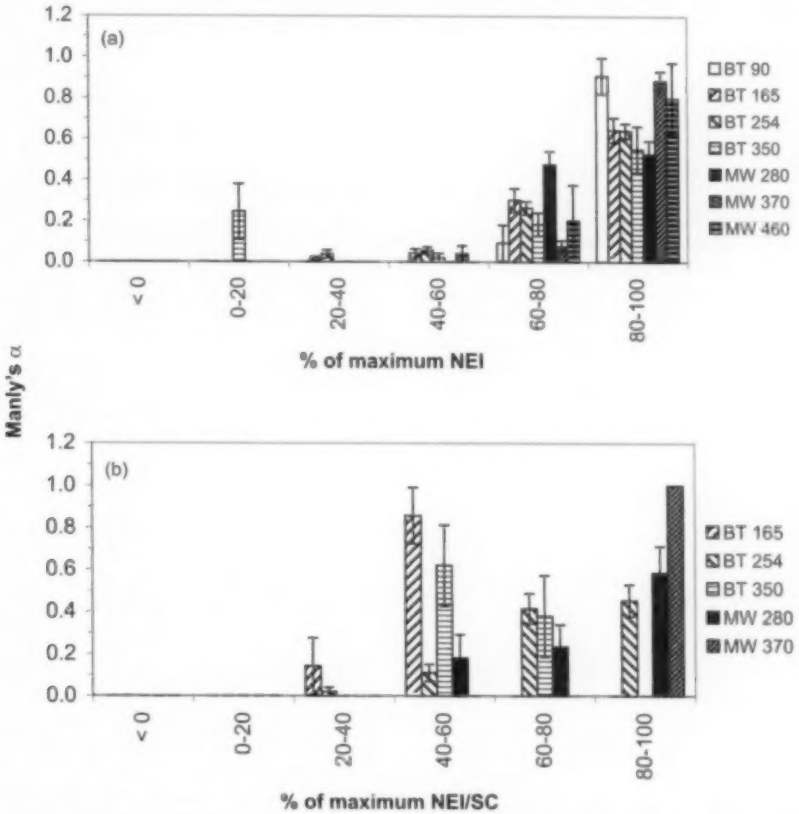

Table 4. Summary of the number of observations (*n*), the dimensionless weighted averages of Manly's preference index (α_{wave}) for net energy intake (NEI) and the ratio of NEI to the swimming cost (SC) at the focal position (NEI/SC), and the associated standard errors (SE) for all size-classes of brown trout (BT) and mountain whitefish (MW).

		Summer					Winter				
Species	Size (mm)	*n*	NEI α_{wave}	±SE	NEI/SC α_{wave}	±SE	*n*	NEI α_{wave}	±SE	NEI/SC α_{wave}	±SE
BT	90	9	77.8	5.6	98.2	1.8					
BT	165	59	75.0	2.1	91.5	1.5	6	100.0	0.0	57.2	2.7
BT	254	175	68.3	2.4	90.2	1.3	48	88.8	4.0	86.1	2.2
BT	350	40	67.6	8.7	75.9	10.1	6	90.0	4.1	67.6	3.9
MW	280	58	69.2	1.7	90.5	1.3	16	66.6	10.6	88.1	4.31
MW	370	82	88.6	3.9	96.9	1.6	9	87.2	3.1	100.0	0.0
MW	460	7	80.0	5.5	96.0	3.5					

river systems with insufficient food resources for fish to achieve maximum daily consumption, fish habitat use should be correlated with measures of NEI. This second scenario corresponds to the time-limited cases discussed in Ydenberg et al. (1992) and Ydenberg and Hurd (1997), where rate maximization is expected. However, even in time-limited/rate-maximizing systems, when choices are available between locations with equivalent NEI, the habitats with the highest NEI/SC ratio should be preferred.

Still, some find the "currency" NEI/SC unsettling because it is different from the two commonly accepted types of currencies, namely, feeding rate (NEI or GEI) and feeding efficiency (GEI/COST). Intuitively, NEI/SC seems similar to efficiency, and thus it is tempting to interpret our result as evidence of efficiency maximization. This is not the case, however. When compared with the spatial pattern of NEI/SC, true efficiency (GEI/COST) has a distinctly different spatial pattern. In fact, GEI/COST, as calculated by our model, does not correlate as well as NEI/SC with the observed fish positions and would predict fish to occupy higher-velocity habitats than those observed.

The physical meaning of NEI/SC remains an issue. We propose that NE/SC simply represents the rate of NEI per the rate of swimming effort at the focal point. In other

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words, 1/SC provides a factor that highlights the locations providing low-effort NEI. It is possible that the effectiveness of NEI/SC is attributable to the fact that the most efficient swimming velocity (V) for a fish is the maxima of V/SC, where SC is a function of V. We will explore this possibility in more detail in a followup publication.

The contradictory winter results for the 165- and 350-mm size-classes of brown trout could result from a number of possibilities. First and most apparently, each of these fish size-classes contained only six fish, which were the two smallest sample sizes in the study. Second, the spatial distributions of fish locations do correlate well with those of NEI/SC; however, they do not fall directly on the few stream positions with the highest values, which could result from measurement error in fish locations or insufficient resolution in the bathymetric/hydraulic data. Small measurement errors in the locations of fish or bathymetric/hydraulic features affect the NEI results less because the NEI distribution is dominated by high NEI values. Of the six fish observed in these size-classes, two 165-mm fish and one 350-mm fish maintained holding positions directly in front of abandoned beaver dams. They were essentially taking advantage of velocity shelter behind a flow separation in front of the hydraulic obstruction, the details of which were not captured in the velocity measurements. The exclusion of these fish from the analysis eliminates the lowest NEI/SC preference for both size-classes (the 20–40% NEI/SC class for 165-mm fish and the 40–60% NEI/SC class for 350-mm fish). This leaves a preference of 1.0 for the 40–60 and 60–80% NEI/SC classes by the 165- and 350-mm size-classes, respectively. In addition, one of the 350-mm fish was observed in large woody debris where velocity measurements were impossible.

Competition, spatial variability of drift, predation, and cover utilization are four important variables that can obviously affect habitat selection but did not appear to be important here. The moderate visibility of the river precluded us from making the extended observations of undisturbed groups of fish that are necessary to isolate competitive interactions or linear dominance hierarchies as documented by other authors (Bachman 1984; Fausch 1984; Hughes 1992). We did not see significant spatial or temporal variability in the limited drift samples that were processed, but particularly in large rivers, such spatial variability may be large and important. It is likely that the effects of predation were not substantial in this study reach because very few fish were observed that were small enough for even the largest size-class of brown trout to consume. Lastly, large woody debris, surface turbulence, terrestrial vegetation, and depth are important habitat features that provide overhead cover. The NEI model does not address predation or the behavioral aspects of cover use by fishes. Of the few fish found in locations with low or moderate NEI/SC, most were in locations with large woody debris cover. In situations where heavy cover utilization affects habitat use, the NEI model may be suitable for estimation of NEI but not for prediction of habitat use.

In summary, we performed the third examination of our mechanistic habitat model for drift-feeding salmonids, which estimates NEI for a chosen size and species of fish, given hydraulic data and drift characteristics. We found that the fish observed in the study reach occupied areas estimated to provide higher than average NEI. We also found that the spatial pattern of the ratio NEI/SC corresponded very well to the observed fish locations. This is especially encouraging because the hydraulics of this study reach were more homogeneous than those of the previous validation study reaches, and therefore, it was more difficult to quantify meaningful differences in habitat quality.

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Hierarchical interpretation of nonlinear relationships linking yellowfin tuna (*Thunnus albacares*) distribution to the environment in the Atlantic Ocean

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Abstract: Using generalized additive models, we show evidence for nonlinear relationships between various hydrological factors and age-structured catch per unit effort of Atlantic yellowfin tuna (*Thunnus albacares*) for two fishing fleets. Catchability effects are distinguished from tuna environmental preference effects in the catch per unit effort variability. With respect to catchability, an important nonlinear effect of local fishing effort is highlighted for each fleet. It is interpreted as resulting from a local overfishing phenomenon of adult yellowfin tuna and from vessel fishing tactics (cooperation/spying). The environmental preferences obtained facilitate the interpretation of the hierarchical spatial distribution and age-dependent movements of the yellowfin population. We show that, on a large spatiotemporal scale (the whole ocean), low salinity is a good predictor of yellowfin habitat. Juveniles are mainly distributed in low-salinity waters (<0.035 kg·kg^{-1}) when adults extend their range to waters of 0.036 kg·kg^{-1}. On a mesoscale, adult population annual reproductive transatlantic displacements are probably driven by temperature and salinity gradients to warm and low-salinity locations that are favorable for juveniles. North–south seasonal movements of the population are clearly related to warmwater seasonal oscillations. On a small scale, ocean thermic stability and gradients of sea surface temperature are important physical factors determining yellowfin concentration.

Résumé : On met en évidence, à l'aide de modèles additifs généralisés, les relations non linéaires existant entre différents facteurs hydrologiques et les captures par unité d'effort en thons albacore (*Thunnus albacares*) de deux flottilles de pêche dans l'Atlantique. L'analyse est structurée en âges. On identifie les effets qui relèvent de la capturabilité et ceux qui relèvent des préférences environnementales des thons. En ce qui concerne la capturabilité, un effet non linéaire important de l'effort de pêche local sur les rendements est mis en évidence pour chacune des flottilles. Il est interprété comme résultant, d'une part d'un phénomène de surexploitation locale des albacores adultes et, d'autre part, des tactiques de pêche des navires (coopération/espionnage). Les préférences environnementales des thons obtenues permettent d'interpréter hiérarchiquement la répartition spatiale et les mouvements de la population d'albacores selon l'âge des animaux. On montre qu'à grande échelle spatio-temporelle (tout l'océan), les faibles salinités sont un bon marqueur de l'habitat des albacores. Les juvéniles sont répartis principalement dans les eaux peu salées (<0,035 kg·kg^{-1}) alors que les adultes étendent leur aire de répartition jusqu'à des eaux de 0,036 kg·kg^{-1}. Aux méso-échelles, les déplacements reproductifs transatlantiques annuels semblent dirigés le long des gradients de température et de salinité vers les eaux chaudes et dessalées favorables aux juvéniles. Les mouvements saisonniers nord–sud de la population sont clairement liés aux oscillations saisonnières des eaux chaudes. A échelle fine, la stabilité thermique de l'océan et l'existence de gradients thermiques de surface sont des facteurs physiques importants de la concentration des albacores.

Introduction

Numerous studies have related fish spatial distribution to environmental factors (Cushing 1982; MacCall 1990; Mann 1993). In the case of highly migratory pelagic species such as tunas, fish distribution is directly linked to the environment. Environmental factors are known to be responsible for fish movements, at least on a large spatial scale (Mendelssohn and Roy 1986; Marsac 1992; Lehodey et al. 1997). Therefore, improving knowledge about environmental factors influencing tuna population dynamics is an objective key issue for management and conservation of tuna stocks

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(Fonteneau 1996). Many studies attempted to describe tuna environmental preferences and to relate their abundance to hydrodynamic features of oceanic waters. But despite the likely nonlinear responses of marine organisms to environmental factors (Kareiva 1990; Mendelssohn 1991), most studies are based on linear analyses (Evans et al. 1981; Marsac 1992).

Yellowfin tuna (*Thunnus albacares*) is a cosmopolitan species mainly distributed in the tropical and subtropical oceanic waters of the three oceans. In the Atlantic Ocean, tagging and catch at size data analyses have shown that yellowfin tuna move massively at different scales in the whole tropical Atlantic Ocean (Bard 1994; Fonteneau 1994). Environmental conditions are probably the main causes driving migration phenomena and massive population movements (Cayré et al. 1988; Cayré 1990). Nevertheless, no clear functional relationship has been determined between yellowfin local abundance, migration patterns, and environment variables.

In this study, we focus on the relationships between the local abundance of yellowfin tuna and different hydrological factors such as salinity, temperature, gradients of sea surface temperature (SST), wind, thermocline depth, currents, and various indices of hydrological stability. For this purpose, generalized additive models (GAMs) are used to link catch per unit effort (CPUE) data for two different fleets (both purse seiners and longliners) to three-dimensional hydrological data from an ocean global circulation model. GAMs are used to characterize the nonlinear relationships. Including in the analysis the effects of auxiliary variables influencing catchability enables us to interpret CPUE data as local abundance indices. Environmental data are then considered both in synchrony with abundance data and with a temporal delay.

Finally, the relationships obtained and their analyses lead us to propose a spatiotemporal hierarchical framework of the processes linking yellowfin tuna to their environment at different scales (small scale (~50 km), mesoscale (~500 km), and macroscale (~5000 km)). From this perspective, observed movement patterns at different scales can clearly be related to the environment.

Materials and methods

Two types of data are used in the present study: fisheries data from the International Commission for the Conservation of Atlantic Tunas (ICCAT) database and hydrological data from the numerical oceanographic model OPA7.1 (Delecluse et al. 1993).

ICCAT catch and effort data

In this study, we use commercial CPUE data from both French, Ivoirian, and Senegalese (FIS) purse seiners for the period 1980–1991 and Japanese longliners for 1980–1993. These two fleets were chosen because their combined catches incorporate the total distribution and size range for this species.

FIS purse seiners target mainly yellowfin, bigeye (*Thunnus obesus*), and skipjack tuna (*Katsuwonus pelamis*) on a wide geographical range but only in the eastern tropical Atlantic. During the period 1980–1991, they kept a relatively stable fishing pattern covering almost the whole yellowfin size spectrum from 30 cm (age 0) to 180 cm (age 5+) (Anonymous 1997). For this fleet, CPUE data are calculated at age (0 to 5+). We use the age decomposition method developed by Gascuel (1994) to age the catch at size data. The method is based on the length at age curve estimated by Gascuel et al. (1992) and assumes a normal distribution of size at age with standard error proportional to mean size increment for each considered cohort. Size to age conversions are computed on a monthly basis for the six spatial zones homogeneous in fish sizes routinely used by ICCAT. FIS purse-seiner CPUE at age are expressed for the six age groups as number of fish per hour of research ($CPUE_{age,zone,time}$ = catches in $number_{age,zone,time}/effort_{zone,time}$). Their spatial resolution is 1 × 1° and their temporal resolution is 15 days. They are used here to characterize yellowfin behavior as a function of their age on a small scale (~50 km) and a mesoscale (~500 km).

Japanese longliners fish the entire Atlantic Ocean, totally covering and probably extending beyond the whole yellowfin distribution range. They fish in deep waters (100–150 m for traditional longlines during the 1980s and down to 300 m for deep longlines during the 1990s) and target mainly adult bigeye and bluefin tuna. Adult yellowfin tuna constituted important by-catches for this fleet during the period under study (Anonymous 1997). Their fishing pattern remained stable during the period 1980–1993, but because they only fish for adult yellowfin between 110 and 170 cm (ages 4 and 5+), we did not age the catches. Japanese longliner CPUE data ($CPUE_{zone,time}$ = catches in $weight_{zone,time}/effort_{zone,time}$) are expressed as tonnes per 1000 hooks. Their spatial resolution is 5 × 5° and their temporal resolution is 30 days. They are used here to characterize adult yellowfin behavior on a mesoscale (~500 km) and a macroscale (~5000 km).

Hydrological data from model OPA7.1

The French Institute of Meteorology (Meteo-France) provided the hydrological data used. These data come from the hydrodynamic model OPA7.1 (Delecluse et al. 1993) developed and validated by the Laboratoire d'Océanographie Dynamique et de Climatologie laboratory (Paris VI). The model is forced by observed winds and assimilates oceanographic data collected routinely, such as temperature profiles and currents, which keep results very close to in situ observations. Simulated data cover the whole tropical Atlantic Ocean from 20°N to 20°S. The model does not work well for coastal zones. The coastal boundaries used in the present analysis correspond to the continental shelf limit and ignore more coastal zones. The spatial resolution is a 0.5 × 0.5° horizontal square grid along 10 depth levels and the temporal resolution is 5 days for the period 1980–1995.

The parameters available for the present study are the temperature of the water column in degrees Celsius (at 5 m assimilated to SST and 15, 25, 36, 51, 70, 90, 110, 130, and 150 m), the depth of the 20°C isotherm in metres (*Z*20), which is usually used to approximate the thermocline depth in the tropical Atlantic Ocean (although arbitrary, this definition gives a measure of thermocline depth consistent with observations), the salinity of the surface water at 5 m depth (kilograms per kilogram), the zonal and meridional components u and v (metres per second) of the water currents at 5 and 51 m depth, and the zonal (x) and meridional (y) components of the pseudo wind stress (newtons per square metre per second), which can be seen as the energy transmitted to the water by the wind.

For a more synthetic description of the hydrological conditions, different parameters were calculated. The water convergence index in cell (x, y), which is based on surface currents, is

$$(1) \qquad \mathrm{conv} = (u_{x-1} - u_{x+1}) + (v_{y-1} - v_{y+1})$$

The current shear index between 5 and 51 m deep, which measures the shear effect exerted on the purse-seine gear, is

$$(2) \qquad \mathrm{CI} = \sqrt{(u_5 - u_{51})^2 + (v_5 - v_{51})^2}$$

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The pseudo wind stress module (newtons per square metre per second) is

$$(3) \qquad |\text{wind}| = \sqrt{x^2 + y^2}$$

The thermocline gradient is $gZ20 = \frac{T_2 - T_1}{Z_2 - Z_1}$ where Z_1 and Z_2 are the first available depths, respectively, just below and just above the depth of the thermocline ($Z20$) and T_1 and T_2 are the corresponding temperatures. The more negative $gZ20$, the stronger the thermocline.

The SST spatial gradient in cell (x, y) is

$$(4) \qquad \text{PSST} = \frac{\partial \text{SST}}{\partial x} + \frac{\partial \text{SST}}{\partial y} \approx \frac{\text{SST}_{x+1} - \text{SST}_{x-1}}{2\Delta x} + \frac{\text{SST}_{y+1} - \text{SST}_{y-1}}{2\Delta y}$$

The SST spatial gradient is calculated with $\Delta x = \Delta y = 0.5°$ and is expressed as degrees Celsius per 1000 km.

Different authors have suggested that past hydrological events are as important as present conditions in explaining tuna distribution (Mendelssohn and Roy 1986; Stretta 1989). Two lags (15 and 30 days) are considered to take into account the local hydrological evolution (cooling or warming) at a given moment. Then, two synthetic water mass warming indices are calculated: $d\text{SST}15 = \text{SST}_t - \text{SST}_{t-15}$ and $d\text{SST}30 = \text{SST}_t - \text{SST}_{t-30}$.

Use of GAMs

GAMs are nonparametric generalizations of multiple linear regression techniques (Hastie and Tibshirani 1990; Swartzman et al. 1992). GAM fitting methods are based on empirical transformations by local smoothing techniques. The general formulation of a GAM is given by

$$(5) \qquad S(Y_j) = \sum_i T_i(X_i(j)) + \varepsilon(j)$$

where Y_j is the response variable for observation j, X_i are the covariates, S is the linked function, T are unknown functions that are nonparametrically estimated using scatterplot smoothers, and ε is a random variable in any distribution of the exponential family.

This recent statistical method is beginning to be commonly used in fishery science (e.g., Mendelssohn and Cury 1987; Cury and Roy 1989; Maravelias 1999). The advantage of GAMs is that they enable multivariate analysis with no a priori assumptions of linearity. In our application, we assume a normal distribution for the log of CPUE + 1. In this case, there is no transformation S (Hastie and Tibshirani 1990; Swartzman et al. 1994) and the expected value of the variable Y is expressed as a sum of smooth functions of the covariates.

We use a logarithmic transformation of the CPUE to normalize the asymmetrical CPUE frequency distribution and because of the a priori multiplicative nature of the processes under study. The general form of the models fitted is

$$(6) \qquad \ln(\text{CPUE} + 1) = \sum_i T_i(X_i) + \sum_j T_j(X_j) + \varepsilon, \quad \varepsilon \approx N(0, \sigma^2)$$

where X_i are the covariates involved in catchability and X_j are the covariates involved in fish density. We use a cubic spline smoother for functions T and the S-Plus software package (©AT&T Bell Laboratories) to fit the models.

Different models are tested to highlight the role of environmental variables on yellowfin density. In each model, all the significant covariates supposed to be involved in catchability are systematically added, associated with the particular environmental variable considered. Because of the important seasonality of the fishery, spatiotemporal effects were systematically included. When they modify the relationship under consideration, significant interaction effects with other environmental variables are added by fitting a spline to the product of the covariates. Following Swartzman et al. (1992), a pseudo R^2 coefficient defined as the fraction of the total deviance explained by the model is systematically computed for each model tested. Although different from the classic R^2, this definition gives a good measure of the explanatory importance of the model. A deviance analysis is then performed to check the significance of each considered factor with an approximate F test computed for each covariate (Hastie and Tibshirani 1990; Venables and Ripley 1994) and to quantify its variability as a fraction of the total variability explained by the model (inertia).

The FIS purse-seine CPUE analysis is done at a resolution of $1 \times 1° \times 15$ days. OPA7.1 environmental data are aggregated at this scale by using a surface weighted mean. The data file obtained contains 35 725 CPUE observations. The model fitted has the following general form:

$$\begin{aligned}(7) \quad \ln(\text{CPUE} + 1) &= s(\text{year}) + s(15 \text{ days} \times \text{ICCAT zone}) \\ &+ s(\text{effort}) + s(\text{wind}) + s(\text{CI}) + s(\text{thermocline depth}) \\ &+ s(\text{thermocline strength}) + s(\text{environmental variable}) \\ &+ s(\text{interaction effects with other variables}) \\ &= A + s(\text{environmental variable}) \\ &+ s(\text{interaction effects with other variables})\end{aligned}$$

where A is the sum of all the transformations supposed to be involved in FIS purse-seine catchability. The year effect is supposed to take into account biomass fluctuations and possible fishing power tendency, as has already been observed for Atlantic yellowfin tuna fisheries (Gascuel et al. 1993). The term (15 days × ICCAT zone) measures a mean spatioseasonal effect in the period considered by fitting a spline to the product of time by the categorical variable zone. It gives equal weight to each zone and 15-day period whatever the fishing fleet distribution. For each effect considered, a general analysis on the whole data set and an analysis by age are done based on the six annual age groups usually used by the ICCAT scientific committee (ages 0 to 5+).

The Japanese longliners CPUE analysis is done at a resolution of $5 \times 5° \times 30$ days. OPA7.1 environmental data are aggregated at this scale. The data file obtained contains 5035 CPUE observations. The model fitted has the following general form:

$$\begin{aligned}(8) \quad \ln(\text{CPUE} + 1) &= s(\text{year}) + s(\text{latitude} \times \text{longitude} \times \text{month}) \\ &+ s(\text{effort}) + s(\text{thermocline depth}) \\ &+ s(\text{environmental variable}) \\ &+ s(\text{interaction effects with other variables}) \\ &= B + s(\text{environmental variable}) \\ &+ s(\text{interaction effects with other variables})\end{aligned}$$

where B is the sum of all the transformations supposed to be involved in Japanese longliner catchability.

The spatiotemporal effect is considered by fitting a three-dimensional spline to the surface defined by the three covariates latitude, longitude, and month. Including the parameters considered to be involved in local catchability in the GAMs allows us to interpret the local CPUE as a local abundance index.

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Table 1. Statistical characteristics of models based on FIS purse-seiner data.

Parameter considered	Model formulation	F test ($p(F)$)	Pseudo R^2	Parameter inertia (%)
Effort	$\ln(\text{CPUE} + 1) = A$	0.0000	0.25	93.0
Z20 general	$\ln(\text{CPUE} + 1) = A + s(\text{SST}):s(Z20)$	0.0000	0.26	2.0
Z20 age 0	$\ln(\text{CPUE} + 1) = A + s(\text{SST}):s(Z20)$	0.0000	0.17	4.9
Z20 age 1	$\ln(\text{CPUE} + 1) = A + s(\text{SST}):s(Z20)$	0.0000	0.15	0.3
Z20 age 2	$\ln(\text{CPUE} + 1) = A + s(\text{SST}):s(Z20)$	0.0000	0.19	0.1
Z20 age 3	$\ln(\text{CPUE} + 1) = A + s(\text{SST}):s(Z20)$	2×10^{-5}	0.20	2.5
Z20 age 4	$\ln(\text{CPUE} + 1) = A + s(\text{SST}):s(Z20)$	0.0000	0.20	2.7
Z20 age 5+	$\ln(\text{CPUE} + 1) = A + s(\text{SST}):s(Z20)$	0.0000	0.19	1.2
gZ20 general	$\ln(\text{CPUE} + 1) = A + s(\text{SST}):s(Z20)$	0.0280	0.25	0.3
Wind	$\ln(\text{CPUE} + 1) = A + s(\text{wind}):s(\text{SST})$	0.0089	0.25	0.005
CI	$\ln(\text{CPUE} + 1) = A$	2.7×10^{-8}	0.25	0.06
Salinity g[al]	$\ln(\text{CPUE} + 1) = A + s(\text{salinity})$	0.0000	0.26	6.8
Salinity age 0	$\ln(\text{CPUE} + 1) = A + s(\text{salinity})$	0.0000	0.19	3.6
Salinity age 1	$\ln(\text{CPUE} + 1) = A + s(\text{salinity})$	0.0000	0.18	17.4
Salinity age 2	$\ln(\text{CPUE} + 1) = A + s(\text{salinity})$	0.0000	0.16	21.2
Salinity age 3	$\ln(\text{CPUE} + 1) = A + s(\text{salinity})$	0.0000	0.19	3.7
Salinity age 4	$\ln(\text{CPUE} + 1) = A + s(\text{salinity})$	0.0000	0.20	0.0
Salinity age 5+	$\ln(\text{CPUE} + 1) = A + s(\text{salinity})$	0.0004	0.19	0.1
SST g[al]	$\ln(\text{CPUE} + 1) = A + s(\text{SST}) + s(\text{SST}):s(Z20)$	0.0002	0.26	2.9
SST age 0	$\ln(\text{CPUE} + 1) = A + s(\text{SST}) + s(\text{SST}):s(Z20)$	0.0000	0.19	0.5
SST age 1	$\ln(\text{CPUE} + 1) = A + s(\text{SST}) + s(\text{SST}):s(Z20)$	0.0000	0.17	4.1
SST age 2	$\ln(\text{CPUE} + 1) = A + s(\text{SST}) + s(\text{SST}):s(Z20)$	0.0000	0.15	2.5
SST age 3	$\ln(\text{CPUE} + 1) = A + s(\text{SST}) + s(\text{SST}):s(Z20)$	0.0000	0.19	1.1
SST age 4	$\ln(\text{CPUE} + 1) = A + s(\text{SST}) + s(\text{SST}):s(Z20)$	0.0000	0.20	1.3
SST age 5+	$\ln(\text{CPUE} + 1) = A + s(\text{SST}) + s(\text{SST}):s(Z20)$	1.3×10^{-5}	0.19	1.0
Convergence	$\ln(\text{CPUE} + 1) = A + s(\text{SST}) + s(\text{convergence}) + s(\text{convergence}):s(\text{PSST}) + s(\text{convergence}):s(Z20)$	0.0400	0.26	0.06
PSST	$\ln(\text{CPUE} + 1) = A + s(\text{PSST}) + s(\text{SST}):s(\text{PSST})$	0.0000	0.26	2.0
*d*SST15	$\ln(\text{CPUE} + 1) = A + s(d\text{SST15}) + s(\text{SST}):s(d\text{SST15})$	0.0000	0.26	0.6
*d*SST30	$\ln(\text{CPUE} + 1) = A + s(d\text{SST30}) + s(\text{SST}):s(d\text{SST30})$	0.0000	0.26	0.4

Results

Parameters involved in fishing power and catchability

Effort

For the FIS purse seiners, as for the Japanese longliners, fishing effort is consistently highly significant and explains the highest part of CPUE variability for all models considered (93% for purse seiners and 48% for longliners (Table 1)). The general relationship between ln(CPUE + 1) and fishing effort is nonlinear for both fleets. For purse seiners, the curve increases rapidly for effort values below a threshold level of around 500 fishing hours. Then, either it slowly increases for ages 0, 1, 2, and 3 or it decreases for ages 4 and 5+ (Fig. 1). For longliners, the curve continuously decreases with a negative exponential shape (Fig. 2).

Thermocline

For FIS purse seiners, the thermocline depth has a clear monotonous negative effect on CPUE during the period considered (Fig. 3). CPUE is higher than the average when the thermocline is less than 50 m deep, and it is lower beyond that point. This effect is constant with age but has a much more important statistical explanatory power for older fishes (ages 3, 4, and 5+) than for young fishes (ages 1 and 2) (Table 1). Removing the interaction effect between SST and thermocline depth enables the thermocline effect to be isolated. Indeed, thermocline depth and SST are not independent variables but are positively correlated. The thermocline gradient ($gZ20$) is less important in terms of the explained variance of the model (Table 1). Nevertheless, its effect is quite clear (Fig. 3). The stronger the thermocline gradient (the more the $gZ20$ index is negative), the higher the purse-seine CPUE.

For Japanese longliners, which are fishing deeper than purse seiners, thermocline depth has a monotonous positive effect (Fig. 2) and explains an important part of the variance of the model (17.4%, cf. Table 2).

Other effects

The wind effect on FIS purse-seine CPUE is consistently highly significant but explains very little of CPUE variability (Table 1). The pseudo wind stress seems to have no negative effects on purse-seiner CPUE until 1000 $N \cdot m^{-2} \cdot s^{-1}$. Beyond this threshold value, the wind has a negative effect on FIS vessel efficiency and catches are lower.

The current shear effect on purse-seine CPUE is significant in most models considered but explains little of the model variance. The curve exhibits a decreasing tendency beyond a threshold level of around 0.4 $m \cdot s^{-1}$.

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Fig. 1. Results of GAM regression (solid lines) of fishing effort on FIS purse-seiner ln(CPUE + 1) for (*a*) age-0 fish and (*b*) age-5+ fish with the 95% SE boundary confidence limits (broken lines) around the covariate effects. Tick marks on the *x*-axis show the location of data points.

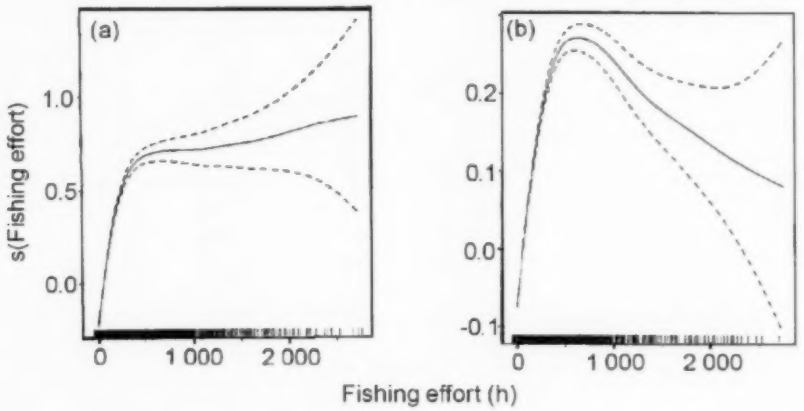
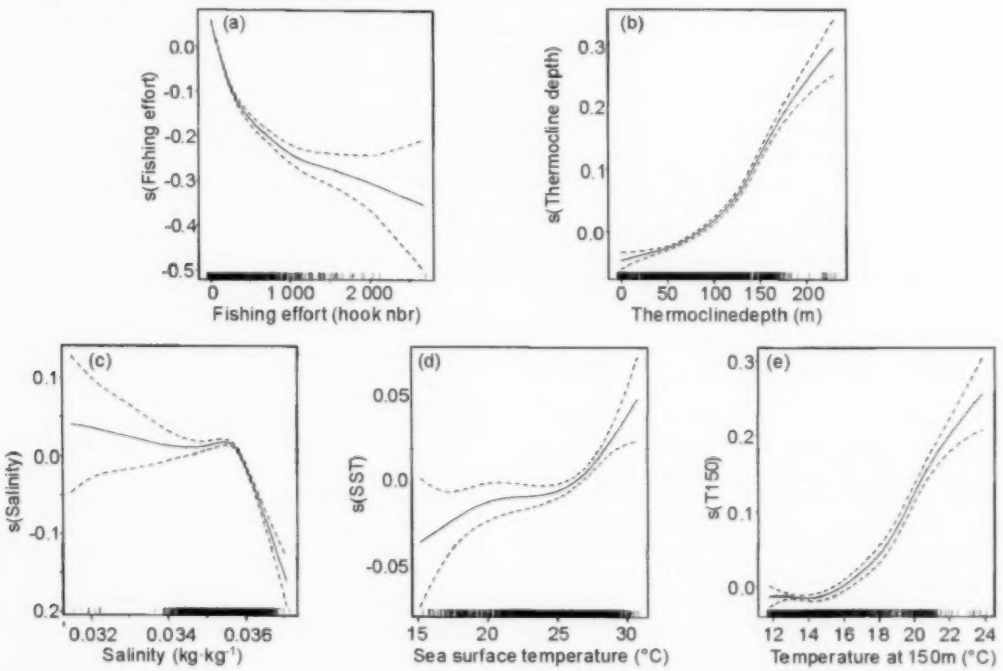


Fig. 2. Results of GAM regression (solid lines) of (*a*) fishing effort, (*b*) thermocline depth, (*c*) salinity, (*d*) SST, and (*d*) temperature at 150 m depth on Japanese longliner ln(CPUE + 1) with the 95% SE boundary confidence limits (broken lines) around the covariate effects. Tick marks on the *x*-axis show the location of data points.



Parameters involved in yellowfin tuna distribution

Salinity

Sea surface salinity has an important explanatory power on FIS purse-seine CPUEs and explains 6.8% of the general model inertia (Table 1). The general relationship between FIS CPUE and surface salinity is characterized by a regularly decreasing curve (Fig. 4). Most catches are made for salinity levels ranging between 0.033 and 0.036 kg·kg^{-1}, but the highest CPUE values occur in low-salinity waters (<0.035 kg·kg^{-1}). For young fishes, the inertia of the model explained by salinity is very high, especially for ages 1 and 2. It decreases at age 3 and is very low for ages 4 and 5 (Table 1; Fig. 5). The relationship with salinity has the same decreasing shape for 0, 1, and 2-year-old fishes. At these ages, CPUEs are higher than the average for salinity lower than 0.035 kg·kg^{-1} and lower for higher salinity values. For older fishes (ages 4 and 5+), low salinity levels below 0.0355 kg·kg^{-1} have no effects on CPUEs. Salinity values higher than 0.036 kg·kg^{-1} seem to have slight negative effects on age-4 and age-5+ CPUEs.

The relationship between longline CPUEs (adult fishes)

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Fig. 3. Results of GAM regression (solid lines) of (*a*) thermocline depth and (*b*) thermocline strength on FIS purse-seiner ln(CPUE + 1), all ages mixed with the 95% SE boundary confidence limits (broken lines) around the covariate effects. Tick marks on the *x*-axis show the location of data points.

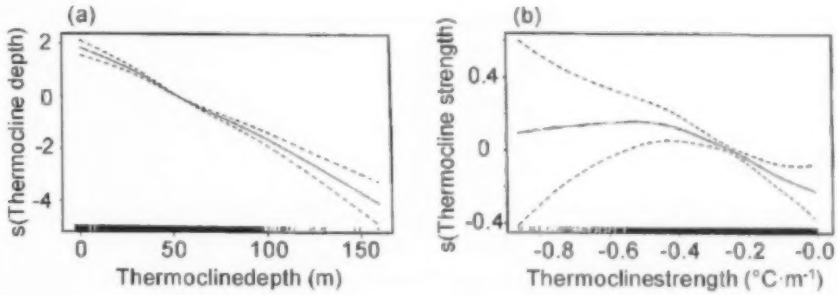

Table 2. Statistical characteristics of models based on Japanese longliner data.

Parameter considered	Model formulation	*F* test ($p(F)$)	Pseudo R^2	Parameter inertia (%)
Effort	ln(CPUE + 1) = *B*	0.0000	0.58	48.26
*Z*20	ln(CPUE + 1) = *B*	0.0000	0.57	18.04
SST	ln(CPUE + 1) = *B* + *s*(SST)	0.0036	0.58	0.94
*T*150	ln(CPUE + 1) = *B* + *s*(*T*150)	0.0000	0.56	9.24
Salinity	ln(CPUE + 1) = *B* + *s*(salinity)	0.0000	0.59	3.74

and surface salinity is highly significant. Like the relationship for adult fishes with purse-seiner CPUEs, there is no effect of low salinity levels until 0.036 kg·kg^{-1}. For values higher than this threshold, salinity seems to be limiting and CPUEs decrease suddenly (Fig. 2).

Temperature

In the general model, SST has an important explanatory power on FIS purse-seine CPUE and explains 2.9% of the model inertia. Most yellowfin catches occur in water temperatures ranging from 25 to 30°C. The general relationship between FIS CPUEs and SST exhibits a constant increase for SST between 22 and 30°C (Fig. 6). In all the models by age, SST is highly significant and explains between 0.5 and 4.1% of the model inertia (Table 1). The inertia of the model explained by SST is high for young fishes, especially for age 1, decreases at age 2, and is low for ages 3, 4, and 5+ (Table 1; Fig. 5). For young fishes (ages 0, 1, and 2), the relationship between FIS CPUEs and SST exhibits a monotonic increasing shape. Older fishes (ages 4 and 5+) tolerate colder waters. The curve characterizing their relationship with SST is dome shaped with a maximum around 27–28°C (Fig. 6).

For Japanese longliners, most catches occur for surface temperatures between 24 and 28°C. Surface temperature effect on CPUE has a low statistical significance for longliners (Table 2). The relationship with temperature at 150 m depth (*T*150) is statistically more significant (Table 2). Most catches occur in the range between *T*150 = 12 and 17°C, and the curve exhibits a linear increase (Fig. 2).

SST gradient

Taking all ages together, the spatial thermic gradient has an important negative effect on fish density. PSST account for 1.13% of the model inertia. Most of the catches are associated with values of PSST between 0 and 25°C·1000 km^{-1}, but higher CPUEs are observed in low-gradient zones for PSST values lower than 10°C·1000 km^{-1} (Fig. 7). Other parameters are significant: the CPUE – convergence index relationship is slightly increasing with a mean positive effect on FIS CPUE for convergence and a mean negative effect for divergence.

Once the interaction effects with SST have been removed (Table 1), the relationship between FIS CPUE and surface temperature evolution (*d*SST15 and *d*SST30) is clear and significant. Surface cooling induces higher CPUEs whereas surface warming induces lower CPUEs. The effect is the same whether a 15- or a 30-day time lag is considered.

Discussion

As for any statistical analysis, results from GAM analysis are sensitive to many factors and must be considered with caution. Furthermore, the environmental data that we use come from an oceanographic numerical model. Even though accurate and well tested, environmental data from the OPA model remain approximations of reality. Thus, the interpretation and consistency of our results with ecological theory remain an important feature to be taken into account.

Use of yellowfin tuna CPUE data as local abundance indices

CPUE is the most often used abundance index in fisheries science. Nevertheless, its use is critical and sets a certain number of problems due to the nonlinearity of the CPUE–abundance relationship. This nonlinearity is generally attributed to the spatial heterogeneity of the resource and to the searching tactics of the fishers (Clark and Mangel 1979; Hilborn and Walters 1992; Gillis et al. 1993). In the case of tuna fisheries, the use of CPUE as local abundance indices is affected by specific phenomena, which are responsible for a

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Fig. 4. Results of GAM regression (solid lines) of salinity on FIS purse-seiners ln(CPUE + 1) for (*a*) age-0, (*b*) age-1, (*c*) age-2, (*d*) age-3, (*e*) age-4, and (*f*) age-5+ fish with the 95% SE boundary confidence limits (broken lines) around the covariate effects. Tick marks on the *x*-axis show the location of data points.

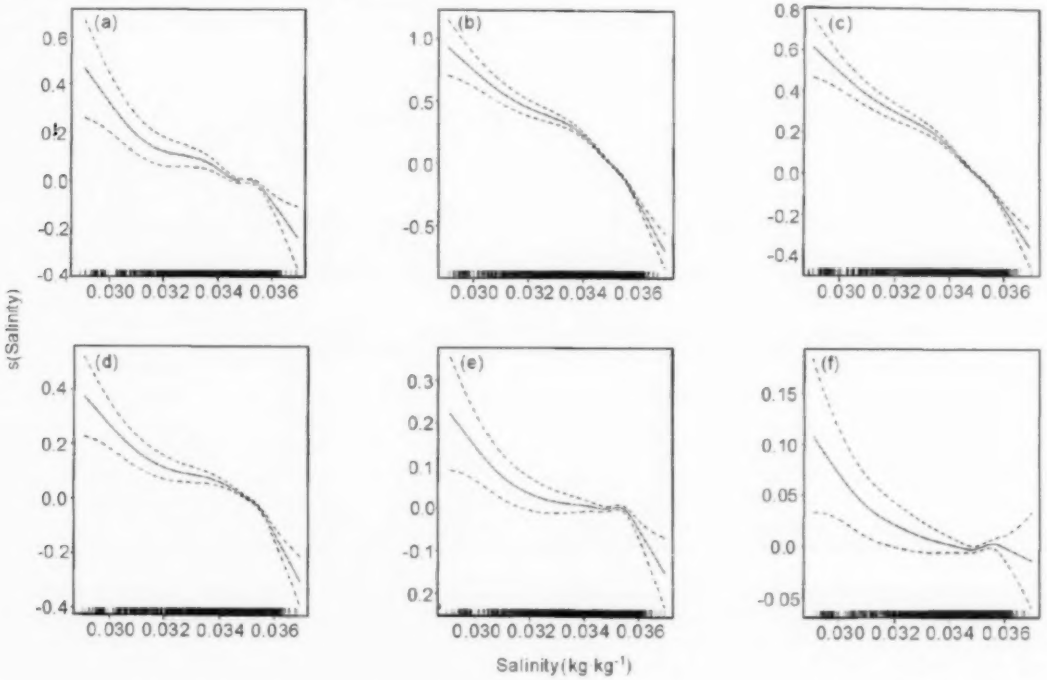
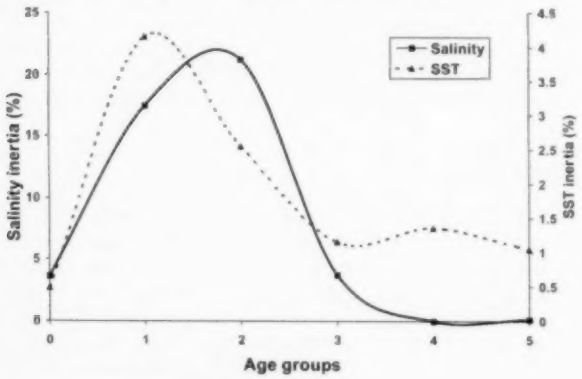


Fig. 5. Inertia explained by salinity (solid line) and SST (broken line) for the six age groups of fish considered (see text).



nonlinearity and a high variability of the local CPUE–effort relationship.

For these reasons, the covariates known to be involved in catchability were systematically added in each model studied. Among those variables, fishing effort was found to have the strongest effect on CPUE for both fleets considered. Maury and Gascuel (2001) have studied with theoretical simulations the nonlinearity of the local CPUE–effort relationship. They have shown that such a nonlinear relationship can be interpreted in terms of vessels' spatial distribution strategies and of cooperation tactics for the increasing part of the curve and of local fish depletion for the decreasing part.

From this perspective, low purse-seine CPUE levels observed for low effort levels could be a consequence of the fishers' experience: the fishers do not explore the zones they know to be scarce in tuna. When purse-seiner effort increases (i.e., the number of searching hours), their catchability rises dramatically because vessels' local fishing power increases due to cooperation (or spying) in locating tuna schools. However, catchability levels off when the total number of hours searched by the whole fleet of vessels is sufficient to locate with a high degree of certainty the greatest concentrations of tuna in the area considered. This threshold is close to 500 h of search per 1 × 1° square during 15 days. For young fishes, which are quite numerous in the environment, local exploitation rates remain relatively low despite the increase of effort and CPUE keeps rising with respect to effort. For older fishes, which are less numerous, the increase in local exploitation rates can be sufficient to lead to local overfishing. Where this occurs, local CPUEs decrease, while local effort keeps on increasing. In the same way, longliners are likely to deplete their fishing zone locally when they are numerous and their yields fall when their local effort increases.

The results that we obtained confirm and emphasize the effects of environmental factors (thermocline depth and strength, wind strength, and currents) on catchability. The negative effect of thermocline depth on purse-seine catchability was also observed in the Pacific Ocean by Inada et al. (1997). It seems to be mainly due to the fact that the thermocline acts as a barrier for yellowfin tuna, especially for young ones that cannot swim in cold waters. When the

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Fig. 6. Results of GAM regression (solid lines) of SST on FIS purse-seiner ln(CPUE + 1) for (*a*) age-0, (*b*) age-1, (*c*) age-2, (*d*) age-3, (*e*) age-4, and (*f*) age-5+ fish with the 95% SE boundary confidence limits (broken lines) around the covariate effects. Tick marks on the *x*-axis show the location of data points.

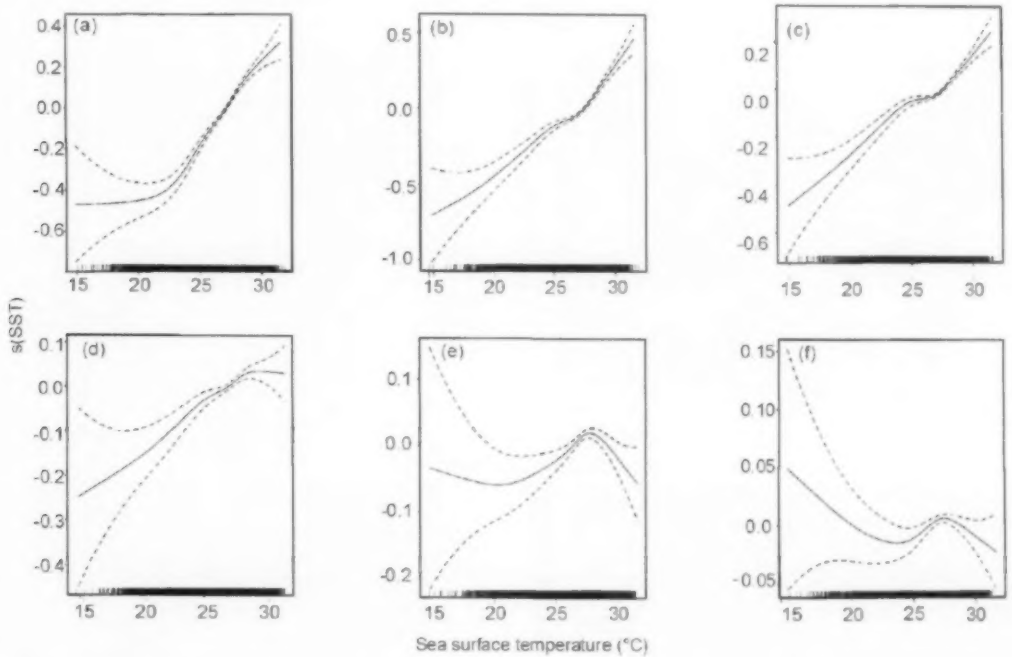
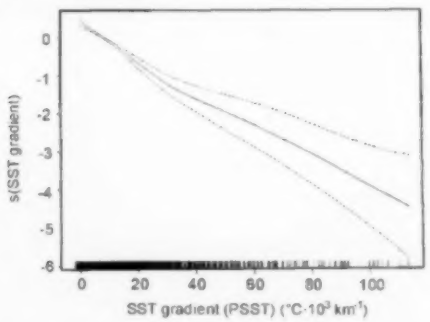


Fig. 7. Results of GAM regression (solid line) of the SST gradient (PSST) on FIS purse-seiner ln(CPUE + 1), all ages mixed, with the 95% standard SE confidence limits (broken lines) around the covariate effects. Tick marks on the *x*-axis show the location of data points.



thermocline is deep or weak, tuna easily escape from the purse-seine nets whereas when it is shallow and strongly marked, tuna are trapped and they cannot escape from the seine (Sharp and Dizon 1978; Evans et al. 1981; Fonteneau 1996). The decrease of catchability for the deeper thermocline seems to be stronger when thermocline depth exceeds 60–70 m, which corresponds approximately to the depth of closing for the seine nets used by FIS purse seiners during the period considered. Such a phenomenon is probably different at present because FIS vessels use much deeper seine nets. Winds have a negative effect for high values. During the period considered, it had been noticed that purse-seine operations were risky when winds exceeded force 4 on the Beaufort scale (Marsac 1992). It is important to take into account the negative impact of shear indices on FIS purse-seine CPUEs. Nevertheless, current shear seems to limit fishing activity for values higher than 0.4 $m \cdot s^{-1}$, which only occur along the equator where countercurrents (mainly the Lomonossov undercurrent) flow to the east, while major surface fluxes are westward. In this zone, purse-seine operations are known to be very risky.

Environmental constraints at different scales

Pelagic fishes live in a variable spatially heterogeneous environment. Because their displacements have an obvious nonrandom component (Sibert and Fournier 1994; Sibert et al. 1996, 1999), their distribution and movements must be constrained by functional relationships (preferendii and constraints) with the environment (Cayré 1990; MacCall 1990; Begon et al. 1996). Such relationships can be local in the case of home range or resource-directed movements or nonlocal in the case of real migrations emancipated from local constraints (Dingle 1996). Our GAM analysis helps to characterize the main relationships between yellowfin tuna distribution, movements, and migrations patterns and the environment at different scales.

At the scale of the whole Atlantic Ocean, water salinity seems to be closely related to the maximal distribution range of the population. Indeed, looking at salinity maps, it is quite clear that zones where the salinity level is lower than 0.036 $kg \cdot kg^{-1}$ correspond to the distribution area of adults, whereas zones where salinity level is lower than 0.035 $kg \cdot kg^{-1}$ correspond to the distribution area of juveniles (Fig. 8). Donguy et al. (1978) noted a clear relationship between

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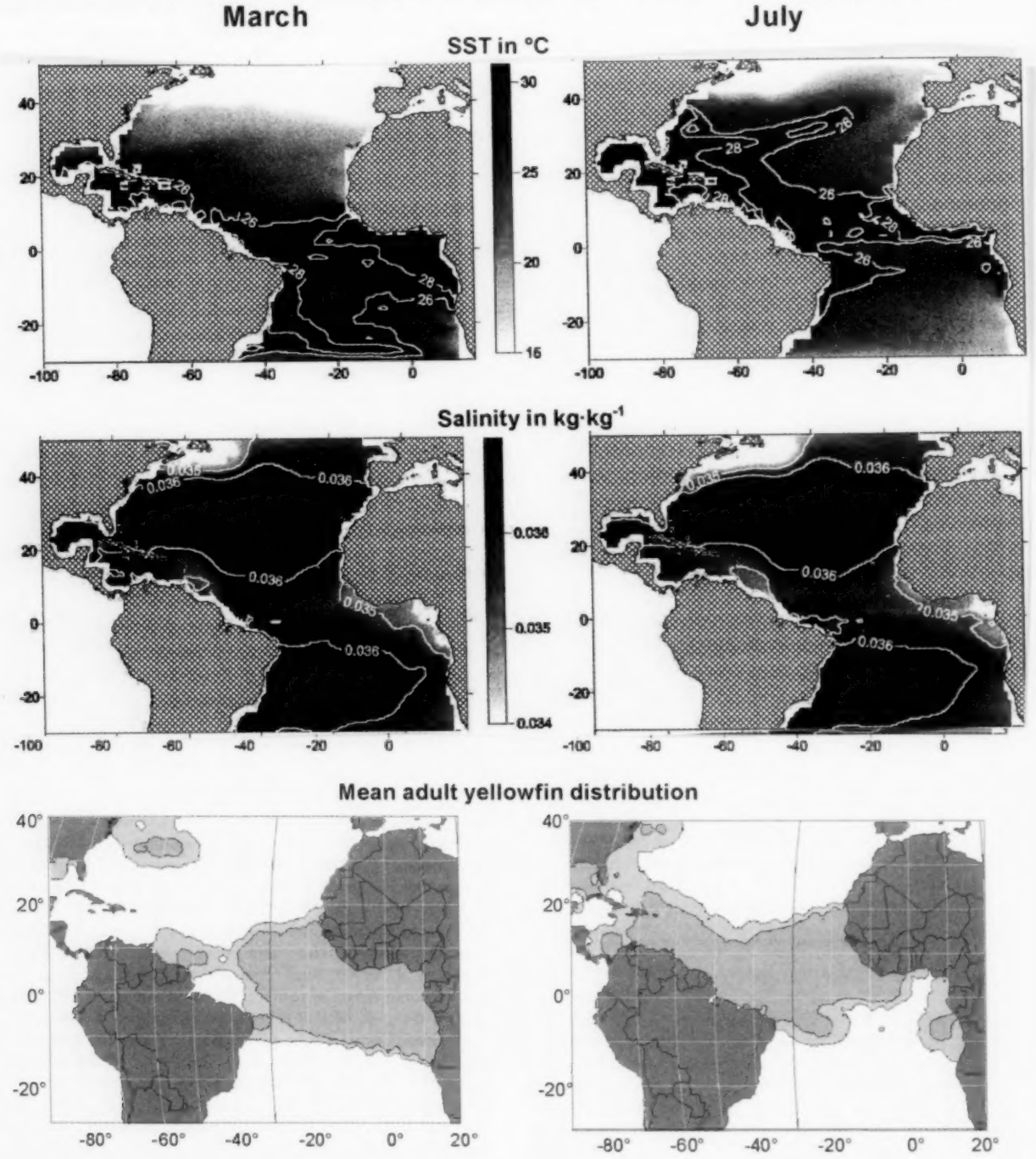
Fig. 8. Comparison between SST maps (top panels) and surface salinity maps (middle panels) in 1991 and mean distribution of the adult yellowfin tuna population estimated by averaging and interpolating all longline catches from 1956 to 1993 (bottom panels).



skipjack catches and salinity in the western Pacific Ocean. Nevertheless, water salinity is generally not considered to be a determinant of yellowfin distribution (Blackburn 1965; Sund et al. 1981; Stretta 1989). By contrast, our results suggest that salinity could be an important marker of the yellowfin distribution range in the Atlantic Ocean. Two reasons could be advocated. For young tuna (ages 0, 1, and 2), salinity could be a marker of favorable trophic areas. Indeed, low salinity levels in the Gulf of Guinea are due to fluvial waters supplies. Such freshwater runoffs contain very high

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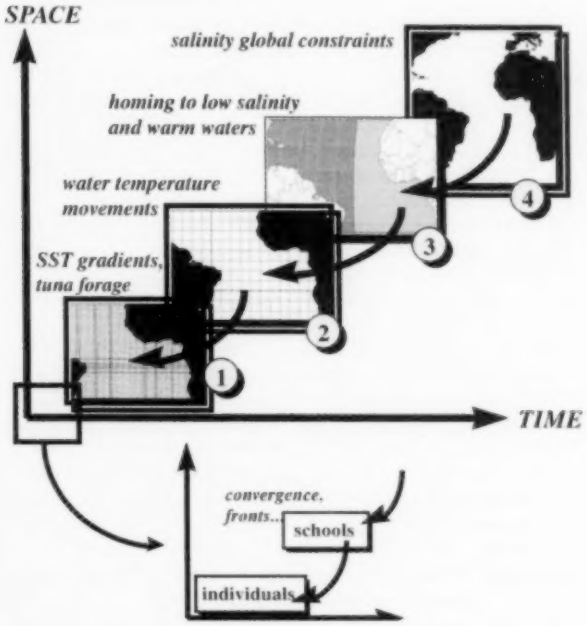
Fig. 9. Hierarchical organization in space and time of the environmental forcing on the yellowfin tuna population in the tropical Atlantic Ocean. Scale 4 corresponds to the maximal distribution range, scale 3 corresponds to the east–west annual displacements, scale 2 corresponds to the north–south seasonal movement of the population, and scale 1 corresponds to the local concentration dynamics; more local scales concern the dynamics of schools and individuals.



nutrient levels, which are generally favorable for young fish nutrition (Bakun 1996). Thus, young tuna forage development is probably related to fluvial enrichment (Caverivière et al. 1976). The other possible explanation is linked to the metabolic cost of osmotic regulation, which could prevent young yellowfin tuna from reaching high salinity levels. Adult yellowfin (ages 3, 4, and 5+) seem to be much more emancipated from salinity constraints at low salinity levels. By contrast, the relationship between salinity and adult yellowfin abundance is clearly significant at high salinity levels. Although oxygen concentration is not considered to be a limiting factor in the Atlantic yellowfin distribution area (Fonteneau and Marcille 1988; Cayré 1990), oxygen needs are very important for pelagic fishes such as tunas (Sharp and Dizon 1978; Sund et al. 1981; Cayre et al. 1988) and could be a determinant of their distribution patterns (Bakun 1996; Fonteneau 1996), especially for adult fishes (Pauly and Moreau 1997). Although not treated in the present work, the relationship with oxygen concentration is probably important, especially for deep longline fisheries. Because oxygen saturation levels decrease with salinity, the apparent link between adult yellowfin distribution and salinity could be related to their high metabolic requirements with respect to dissolved oxygen.

At a large scale (5000 km), the adult yellowfin population moves massively across the Atlantic Ocean. During the fourth and first quarters, fish converge from their entire distribution area towards the main spawning ground in the Gulf of Guinea (Capisano and Fonteneau 1991; Bard 1994; Fonteneau 1994). After breeding, adults disperse in the whole Atlantic Ocean to feed in the distribution area characterized by salinity, whereas juveniles stay in the lower-salinity waters of the Gulf of Guinea. In this way, east–west adult reproductive displacements take place inside the maximum distribution range related to salinity and are probably directly related to warmwater seasonal oscillations (Fig. 8). But because adults are less constrained by salinity and temperature than are juveniles, such migrations could be, at least partly, driven by a homing behavior to the low-salinity and high-temperature locations favorable for larvae and the survival of juveniles (Caverivière et al. 1976). It could be an example of the obstinate nature in philopatric migrations as hypothesized from an evolutionary point of view by Cury (1994).

At a medium scale (1000 km), the fish population moves seasonally along a north–south axis along the African coast for the juveniles and in the open ocean for adults (Bard 1994; Foucher 1994) (Fig. 8). Such seasonal migrations should be related to the annual cycle of the meridional oscillation of warm waters. Our results show that adults stay preferentially in zones where surface waters are in the range of 26–29°C and where deeper waters (150 m depth) are warmer than 15°C. Juveniles, whose temperature regulatory system is not yet effective (Cayré 1990), stay in surface waters in areas where SST is higher than 27°C. Preadults (ages 2 and 3) also follow warm waters around 27°C but seem to be more tolerant of temperature variations. Warmwater preferences are well known for tunas. They allow tunas to warm their body after a deep dive in cold waters (Sharp and Dizon 1978; Holland et al. 1992; Fonteneau 1996).

At a local level (100 km), tunas seem to be influenced by both local hydrological and biological features, which may concentrate them. Among the various factors, which may act at this scale on yellowfin concentration, tuna prey distribution must be fundamental. However, the high variability of the spatial distribution of the various small pelagic fish and cephalopod targeted by tunas (Cayré et al. 1988; Roger 1990) is not directly linked to local enrichment processes such as upwelling, dome, or divergence (Fonteneau and Marcille 1988). Indeed, a time lag is necessary to relate primary production to the concentration of tuna forage. Without considering displacements by oceanic currents, Garcia and Cota (1996*a*, 1996*b*) have found a correlation between phytoplankton production and yellowfin CPUE with a time delay of between 3 and 5 months in the Gulf of Mexico. By using a secondary production index estimated by advection–diffusion of primary production data following marine currents, Lehodey et al. (1998) and Maury (1998) suggested that a 3- to 7-months time lag could separate phytoplankton peaks from the tuna prey's highest concentration.

Independently of biological factors, our results also suggest physical causes of local fish concentration such as the spatial stability of water masses. Because yellowfin follow the temperature gradient to reach warm waters, they do not stay within the high horizontal gradient *s*(PSST). When water temperature exhibits rapid changes, fishes are pushed by the unfavorable cooling side of the area. Associated with secondary production patches and local effects of seamonts

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or islands (Fonteneau 1991, 1996), they could be responsible for local (100 km) yellowfin concentrations.

More local scales (100 m to 10 km), particularly concerning individuals and schools dynamics, are only considered through catchability in the present study. Nevertheless, they are probably at the center of important variability in fish abundance. Factors probably involved at these very local levels are the presence of floating objects and the existence of small-scale hydrological events such as local fronts or convergences. Such hydrological features may control tuna forage concentration as convergence does, for instance, by concentrating floating objects (Bakun 1996). Such a behavior, well known by the fishers that follow convergence lines to locate tuna schools, is probably too local to be detected accurately by our $1 \times 1° \times 15$ days analysis.

Because environmental constraints and forcing have various spatiotemporal frequencies, the population level may also be considered as hierarchically organized in space and time. In the case of the Atlantic yellowfin tuna population distribution, different hierarchically organized scales can be distinguished (Fonteneau 1996). At each of these levels of variability, we can associate an environmental factor, as pointed out by our results (Fig. 9). Finally, the complex dynamics of the yellowfin tuna distribution and movements emerge from the interaction of these different processes occurring at different scales.

Beyond the help that it can bring to fishers, the knowledge of the nonlinear environmental processes and their hierarchical organization is of critical importance for fisheries science and fisheries management faced with environmental variability at different scales. One of the main recommendations emerging from the Rio conference on sustainable development was to learn to think from the local to the global scale and vice versa. By articulating global and local scales, a hierarchical understanding of ecosystems is a means of going in this direction.

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Linking shrimp assemblages with rates of detrital processing along an elevational gradient in a tropical stream

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Abstract: We experimentally excluded freshwater shrimp assemblages (Atyidae, Xiphocarididae, and Palaemonidae) to examine their effects on detrital processing and benthic insect biomass at three sites along an elevational gradient in a tropical stream in Puerto Rico. We also determined which shrimp taxon was responsible for leaf decay in a subsequent laboratory experiment. At the high-elevation site, the shrimp assemblage was dominated by *Atya* spp. and *Xiphocaris elongata*, and leaf decay rates were significantly faster in the presence of shrimps than in their absence. Laboratory experiments showed that this was primarily due to direct consumption of leaves by *Xiphocaris*. Shrimps had no effect on leaf decay rates at mid- and low-elevation sites where there were higher proportions of *Macrobrachium* spp. shrimps (which prey on *Xiphocaris*). Laboratory experiments showed that *Xiphocaris* consumed significantly less leaf material and experienced significantly higher mortality in the presence of *Macrobrachium*. Shrimp exclusion resulted in significantly less and significantly more insect biomass at the high- and low-elevation sites, respectively; no difference was found at the mid-elevation site. Insects played a minor role in leaf decay. Results show a strong linkage between shrimp assemblages and rates of detrital decay and illustrate the importance of conducting experiments at multiple sites.

Résumé : Nous avons artificiellement exclu les communautés de crevettes d'eau douce (Atyidae, Xiphocarididae et Palaemonidae) de trois sites le long d'un gradient altitudinal dans un cours d'eau tropical à Porto Rico dans le but de d'étudier leur effet sur la transformation du détritus et la biomasse des insectes benthiques. Dans une expérience ultérieure en laboratoire, nous avons déterminé quels taxons de crevettes étaient responsables de la décomposition des feuilles. Au site de haute altitude, le peuplement de crevettes était dominé par des *Atya* spp. et par *Xiphocaris elongata*; la décomposition des feuilles s'y opérait significativement plus vite en présence des crevettes qu'en leur absence. En laboratoire, il a été démontré que cela était dû à une consommation directe des feuilles par *Xiphocaris*. Les crevettes n'avaient pas d'effet sur le taux de décomposition des feuilles aux sites d'altitudes moyenne et basse, où il y avait une plus forte proportion de *Macrobrachium* spp., des prédateurs de *Xiphocaris*. En laboratoire, *Xiphocaris* consommait significativement moins de feuilles en présence de *Macrobrachium* et sa mortalité était significativement plus élevée. L'exclusion des crevettes a entraîné une réduction significative de la biomasse des insectes au site de haute altitude et une augmentation de cette biomasse en basse altitude; en altitude moyenne, il n'y a eu aucun changement. Les insectes ne jouaient qu'un rôle mineur dans la décomposition des feuilles. Nos résultats démontrent l'existence d'une forte relation entre les communautés de crevettes et les taux de décomposition du détritus et illustrent l'importance de mener des expériences simultanément à plusieurs sites.

[Traduit par la Rédaction]

Introduction

Understanding the roles of species in ecosystem processes such as primary production, decomposition, and nutrient cycling can provide important insights into the functioning of ecosystems as well as guide managers in designing conservation strategies (Jones and Lawton 1995). Recently, ecologists have emphasized the importance of incorporating spatial heterogeneity into experiments to explore not only whether certain species play key roles, but also under which ecological conditions they assume these roles (Hunter and Price 1992; Polis et al. 1996). Therefore, to understand species effects on ecosystem processes more fully, it is necessary to incorporate spatial heterogeneity because links between species and ecosystem function may vary with ecological conditions. In lotic ecosystems, most experimental studies are conducted at a single site and therefore are unable to address the importance of certain species beyond the context of a specific stream reach (e.g., Pringle et al. 1993). Would the role of these species change if the study was conducted a few kilometres up or downstream?

In this study, we examined the role of freshwater shrimps (Decapoda: Caridea) in determining rates of leaf litter decay

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and composition and biomass of insect assemblages at three sites along an elevational gradient in a Puerto Rican stream. Previous studies in the headwaters of this stream demonstrated that shrimps affect insect and algal community composition as well as sediment cover and the quantity and quality of fine benthic organic matter (Pringle and Blake 1994; Pringle 1996; Pringle et al. 1999). However, community composition and abiotic conditions change with elevation (Bhajan et al. 1978), and this may affect the relative roles of different macroconsumers. Understanding the role of shrimps at lower-elevation sites is important, given their migratory life cycle and the nature of recent anthropogenic disturbances. All of the shrimp species in these rivers have diadromous life cycles and migrate between upland freshwater and downstream estuaries during their lives (Covich and McDowell 1996; March et al. 1998; Benstead et al. 2000). Recent anthropogenic disturbances such as damming and water withdrawal have affected distributions of adult shrimps and larval survival rates (Holmquist et al. 1998; Benstead et al. 1999). Thus, understanding the role that shrimps play along an elevational gradient has both ecological and resource management implications.

Several studies have experimentally examined effects of large omnivorous crustaceans (shrimps and crayfishes) on insects and algae in streams (e.g., Pringle and Blake 1994; Charlebois and Lamberti 1996). These studies demonstrated that shrimps and crayfishes could have direct effects via consumption as well as indirect effects via trophic cascades and bioturbation. Considerably less is known about effects of shrimps and crayfishes in detrital food webs, specifically their effects on leaf litter and associated insects. Detrital material from terrestrial vegetation is the dominant energy source in many streams, and within-stream decay of terrestrial leaves is a well-studied process (see reviews by Webster and Benfield 1986; Boulton and Boon 1991). However, few studies have examined experimentally their contribution to leaf litter decay (but see Parkyn et al. 1997; Rosemond et al. 1998; Crowl et al. 2001) or how it varies along stream continua.

In this study, we combined the use of an in situ manipulation of shrimps at three sites along an elevational gradient in a tropical stream with a laboratory experiment to understand the role of shrimps in an ecosystem process and benthic community structure. Specifically, we addressed the following two questions. (*i*) Do different shrimp assemblages along an elevational gradient affect rate of leaf decay and insect community composition differently along this gradient? (*ii*) Which shrimp taxa are most important in determining rates of leaf decay?

Materials and methods

Study site

This study was conducted in the Río Espíritu Santo river system, which drains the northern part of the Luquillo Experimental Forest and surrounding lowlands in northeastern Puerto Rico. The physical structure of the Río Espíritu Santo changes dramatically from high-elevation headwaters to river mouth. High-elevation tributaries (>200 m above sea level) are characterized by alternating pools and cascades. Substrata are composed of large boulders with interstitial cobble and gravel. A large waterfall (>10 m), at approximately 200 m above sea level, acts as a barrier to most fishes. At mid-elevation sites (70–200 m above sea level), small cascades are less frequent, and riffles composed of small boulders and cobble are common. Low-elevation sites (<70 m above sea level) are characterized by large deep runs and cobble–gravel riffles.

Land use also changes along this elevational gradient. Tabonuco (*Dacryodes excelsa*) forest extends from 600 m to the Luquillo Experimental Forest boundary located at 90 m. Below the boundary, land use changes to urban and pasture; however, the riparian zone is predominantly forested. Allochthonous inputs enter the river system throughout the year, although two peaks in leaf input occur in the tabonuco forest (from March to May and September to October (Covich and McDowell 1996)).

Ten species of omnivorous freshwater shrimps inhabit rivers of the Luquillo Experimental Forest. The palaemonid shrimps *Macrobrachium carcinus* (L.), *M. faustinum* (De Saussure), *M. crenulatum* Holthuis, *M. acanthurus* (Wiegmann), and *M. heterochirus* (Wiegmann) can reach sizes >230 mm in length and consume algae, macrophytes, decomposing leaf litter, insects, molluscs, small fish, and other shrimps (Covich and McDowell 1996). The atyid shrimps, represented by *Atya lanipes* Holthuis, *A. scabra* (Leach), *A. innocous* (Herbst), and *Micratya poeyi* (Guérin-Méneville), consume periphyton, small sessile insects, and leaf matter by brushing with their modified cheliped fans (Pringle et al. 1993; Pringle 1996). They use these same cheliped fans to filter feed when flow conditions are suitable (Covich 1988). *Xiphocaris elongata* (Guérin-Méneville) consumes leaf matter, insects, small flowers, and fruit using tiny pincers. No information is available on food preference for any of these shrimp species. All of these shrimp species undergo extended larval development and are amphidromous (Covich and McDowell 1996); adult females release planktonic larvae that drift downstream from freshwater habitat to the estuary (March et al. 1998). Larvae spend 50–110 days in the estuary before migrating back upstream as metamorphosed postlarvae (Hunte 1979; Benstead et al. 2000).

The remainder of the aquatic fauna consists of seven species of fishes, insects, snails, and one species of crab. Of the fishes, only the green stream goby *Sicydium plumieri* is found above the high waterfall at 200 m. The insect assemblage is depauperate when compared with continental tropical streams (Buzby 1998) and is dominated by sessile chironomids and mobile baetid and leptophlebiid mayflies (Pringle et al. 1993; Buzby 1998). Three species of freshwater snails, *Neritina punctulata*, *N. clenchi*, and *Thiara granifera*, inhabit mid- and low-elevation sites. These snails graze periphyton. The freshwater crab *Epilobocera sinuatifrons* is omnivorous and consumes insects, shrimps, fruit, and seeds (Covich and McDowell 1996).

In situ experiment

The field experiment was conducted from 18 July to 17 August 1996. The high-elevation site was at 300 m in the Sonadora, a second-order tributary of the Espíritu Santo. The mid-elevation site was located approximately 3.1 km downstream at 90 m in a third-order mainstem reach. The low-elevation site was a side channel of the fourth-order mainstem at 10 m, approximately 4.2 km downstream from the mid-elevation site. At each site, five depositional areas were chosen close to the stream bank where leaves accumulated naturally. In each depositional area, two quadrats were anchored to the stream bottom using rocks from the stream and cable ties. Quadrats were square templates (45 × 45 cm) made of 19-mm chlorinated polyvinyl chloride tubing and contained an inner and outer square of uninsulated 12-gauge copper wire. A coin flip determined which one would be electrified (shrimp exclusion) or a control (shrimp access). Each electric quadrat was connected to a solar-powered fence charger (Parker-McCrory, 12-V) by attaching 12-gauge copper wire from the inner square to the power source and from the outer square to the ground. This method excluded fishes and shrimps but not insects and snails. Leaf packs were

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made from air-dried freshly fallen green leaves of *Cecropia schreberiana* cut into smaller pieces, weighed to approximately 5 g, held together with a metal binder clip, and tethered inside the quadrat. *Cecropia schreberiana* was used because it is common in the tabonuco forest, especially along stream banks (Brokaw 1998). One leaf pack was sampled randomly from each replicate treatment on days 1, 3, 6, 9, 12, 20, and 25. We sampled the remaining three leaf packs on day 30. We also sampled 30 leaf packs on day 0 to obtain an air-dried to oven-dried conversion factor, which was used to adjust the original air-dried weights of the leaf packs. After collection with a fine-mesh net, leaf packs were oven-dried at 50°C for 24 h and weighed to the nearest 0.01 g. Leaf decay rates (k) were calculated for each treatment replicate by regressing ln(% leaf mass remaining) against elapsed days, starting with day 1; k is the negative slope of the regression (Benfield 1996). We used an average percent remaining of the three leaf packs sampled from each treatment replicate for the day-30 sample. Leaf decay rates were compared between treatments and among sites using a two-way analysis of variance (ANOVA). Because we found a significant site × treatment interaction, we examined differences in leaf decay rate between treatments at each site separately using one-way ANOVAs. A one-way ANOVA was also used to compare decay rates among sites in the absence of macroconsumers. Because of a large spate on day 27, statistical analyses were conducted both with and without data from day 30.

Insects found on leaf packs on days 6, 9, and 20 were preserved in 70% ethanol, identified to family or genus, and measured to the nearest millimetre. Insect biomass per leaf pack was calculated using published family-level length–mass relationships (Benke et al. 1999). If a length–mass relationship was not available for a family, we used one from a taxon with a similar shape. Insects were assigned to a functional feeding group based on literature and mouthpart morphology (Merritt and Cummins 1984; Buzby 1998). Differences in total insect biomass, biomass of shredders, and the biomass of the two most common groups (Ephemeroptera and Chironomidae) were compared between treatments and among sites using a two-way repeated measures ANOVA on $\log(x + 1)$-transformed data. If a significant site × treatment interaction was found, we examined each site individually using a one-way repeated measures ANOVA. Snails found on leaf packs were counted and compared using two-way repeated measures ANOVA.

We electroshocked a 2-m^2 area around each treatment replicate with a backpack electroshocker at the conclusion of the experiment. Shrimps were identified to genus (note: *M. poeyi* was lumped in with *Atya* spp.) and the carapace length of shrimps was measured to the nearest millimetre. Shrimp abundance was converted to biomass using length–mass relationships (March 2000). We also conducted 2-min observations of each quadrat on six different dates. Observations were conducted from nearby large boulders so that fish and shrimp behavior were not affected. Shrimps were identified to genus when visibility allowed.

Water depth and velocity were measured at base flow on two days at the conclusion of the experiment using a ruler and a Marsh–McBirney digital flowmeter, respectively. Water velocity was measured inside the quadrat where leaf packs were positioned. The range of water temperatures at each site was measured using submerged min–max thermometers.

Discharge values were obtained from two U.S. Geological Survey gauges in the Espíritu Santo, located just below our high-elevation site and between the mid- and low-elevation sites. Discharge at each site was then estimated using methods in March et al. (1998).

Laboratory experiment

To determine which shrimp taxa were responsible for effects on leaf decay observed in the in situ experiment, we conducted a laboratory experiment using aquaria during August 1998. Each 11.4-L rectangular aquarium (40 × 27 × 13.5 cm) contained cleaned natural cobble–pebble substrate, a flow pump (Aquarium Systems Maxi-jet 500), and a 5.00 ± 0.37 g leaf pack of air-dried freshly fallen *C. schreberiana*. We used a factorial design with three genera of shrimp and a control. The treatments were as follows: *Atya* only (A), *Xiphocaris* only (X), *Macrobrachium* only (M), every possible combination of these genera (AX, AM, XM, and XMA), and a control with no shrimp. Each treatment was replicated three times and each aquarium contained six shrimps divided evenly among the genera. Shrimps were identified to genus, measured to the nearest millimetre (carapace length), and wet-weighed to the nearest 0.1 g (Table 1). Any dead shrimps were identified and replaced daily. Filtered stream water in each aquarium was changed at least every other day. Leaf packs were removed after 17 days, oven-dried at 50°C for 24 h, and weighed to the nearest 0.01 g. Leaf mass consumed and the number and genus of shrimp mortalities were compared among treatments using Tukey–Kramer HSD (honestly significant difference) tests with significance set at 0.05. To test for interactions among shrimp genera in aquaria, we compared predicted leaf mass consumed (based on single-taxon treatments) with actual leaf mass consumed using a t test. Predicted leaf mass consumed for each treatment was calculated by multiplying the leaf mass consumed per gram of shrimp of each taxon alone by the biomass (wet weight (grams)) of each shrimp taxon in each treatment.

Results

In situ experiment

Shrimp assemblages

Shrimp assemblage composition differed among sites. At the high-elevation site, *Xiphocaris* and *Atya* shrimps were observed to enter the control treatment in approximately equal numbers (Table 2). However, based on electroshocking, the shrimp assemblage was dominated by *Atya* followed by *Xiphocaris* and *Macrobrachium* in terms of both abundance and biomass (Table 3). No fishes were observed in the control treatment or sampled with electroshocking at the high-elevation site. At the mid-elevation site, shrimps observed in the control treatment were mostly *Xiphocaris*, some *Macrobrachium*, and no *Atya* (Table 2). However, *Macrobrachium* comprised the majority of the shrimp assemblage based on electroshocking, with fewer *Xiphocaris* and few small *Atya* (Table 3). Again, no fishes were observed in the controls at this site, but one fish (*Xiphophorus helleri*) was sampled with electroshocking. At the low-elevation site, *Macrobrachium* shrimps comprised the majority of the shrimp assemblage observed in controls and sampled by electroshocking (Tables 2 and 3). Again, no fishes were observed in controls; however, one individual each of three fish species (*Anguilla rostrata*, an unidentified gobiid, and *X. helleri*) and one crab (*E. sinuatifrons*) were sampled by electroshocking.

The mean size of individuals within each shrimp taxon decreased in a downstream direction (Fig. 1). Medium and large *Atya* were only found at the high-elevation site. Large *Xiphocaris* were found at the high- and mid-elevation sites, while large *Macrobrachium* were found at all sites (Fig. 1).

Leaf decay

Effects of shrimps on leaf decay varied significantly among sites ($F_{2,24} = 8.47$, $P < 0.01$) and were therefore examined separately (Fig. 2). At the high-elevation site, leaf

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Table 1. Carapace lengths and biomass of individuals of each taxon used in the laboratory experiment.

	Carapace length (mm)		Wet weight (g)	
Taxon	Range	Mean ± 1 SE	Range	Mean ± 1 SE
Atya	8–16	11.9±0.3	0.8–4.3	2.1±0.14
Macrobrachium	8–19	12.6±0.3	0.7–4.9	1.9±0.11
Xiphocaris	7–13	10.0±0.1	0.4–2.0	1.0±0.04

decay rate was significantly greater in the presence of shrimps than in their absence ($F_{1,8} = 8.23$, $P = 0.02$). In contrast, there was no significant difference in leaf decay rate between treatments at the mid-elevation ($F_{1,8} = 3.58$, $P = 0.10$) or low-elevation site ($F_{1,8} = 2.67$, $P = 0.14$). Excluding day 30 from statistical analyses did not affect significance.

Effects of shrimps on insects

The effect of shrimps on insect biomass on leaf packs also varied significantly among sites ($F_{2,24} = 6.04$, $P < 0.01$) (Fig. 3). At the high-elevation site, insect biomass was significantly greater in the control treatment ($F_{1,8} = 8.23$, $P = 0.02$). In contrast, the effect was opposite at the low-elevation site; insect biomass was significantly higher in the shrimp-exclusion treatment ($F_{1,8} = 6.75$, $P = 0.03$). The effect of shrimps on insect biomass at the mid-elevation site was not significant.

Insect shredders, ephemeropterans, and chironomids comprised 92% of the insect biomass over all sites. Insect shredders were rare and were composed of only two taxa: *Phylloicus* sp. (Trichoptera: Calamoceratidae) and *Phanocerus* sp.(Coleoptera: Elmidae). There was no effect of shrimps, site, or an interaction between the two on shredder biomass (Fig. 4).

Ephemeroptera were mostly leptophlebiids, with some baetids and caenids. The effect of shrimps on Ephemeroptera biomass in leaf packs also varied significantly among sites ($F_{2,24} = 5.70$, $P < 0.01$). When we examined each site separately, shrimp effects were significant only at the high-elevation site where mayfly biomass was greater in the presence of shrimps ($F_{1,8} = 12.40$, $P = 0.008$) (Fig. 4). Shrimps significantly reduced biomass of chironomids at all sites and there was a greater biomass of chironomids at the mid-elevation site (Fig. 4). Snails were only found at mid- and low-elevation sites and there was no shrimp effect on snail abundance ($F_{1,24} = 0.18$, $P = 0.68$).

Abiotic factors

At the high-elevation site, temperature ranged from 21 to 23°C, while water velocity averaged 0.015 $m \cdot s^{-1}$. The mid-elevation site experienced temperatures of 23–28°C and higher flow velocity (0.024 $m \cdot s^{-1}$). Mean flow rate at the low-elevation site was 0.043 $m \cdot s^{-1}$. Temperature at this site ranged from 23 to 29°C. High-discharge events occurred during the study on days 2, 8, 17, and 27 (Fig. 2). These discharge events did not seem to have a dramatic effect on leaf decay with the exception of the storm on day 27 (Fig. 2). Despite differences in abiotic conditions, leaf decay rates were not different among sites in the shrimp-exclusion treatment ($F_{2,14} = 1.13$, $P = 0.35$).

Laboratory experiment

Leaves in the X and AX treatments lost significantly more mass than in all other treatments, except when all three genera were combined (XMA) (Fig. 5). In contrast, the amount of leaf mass lost from leaf packs in treatments A, M, AM, and XM was not significantly different from that in the control (Fig. 5).

In treatments with multiple genera of shrimp, we found differences between observed and predicted leaf mass loss based on leaf mass loss in single-taxon treatments (Fig. 6). The observed leaf mass loss was less than predicted in each treatment that contained *Macrobrachium*; however, this was only significant in treatment XM ($P = 0.04$) (Fig. 6), indicating that *Xiphocaris* breaks down less leaf mass with *Macrobrachium* present than when alone. Leaf mass loss in treatment AX was greater than predicted than when each genus was alone ($P = 0.04$) (Fig. 6), suggesting a positive interaction between *Atya* and *Xiphocaris*.

There were also differences in shrimp mortality among treatments (Fig. 7). Mortality of *Xiphocaris* was significantly higher in treatments with *Macrobrachium* (XM and XMA) than when alone (X) or with *Atya* (AX) (Tukey–Kramer, $P < 0.05$) (Fig. 7). There was also higher mortality of *Atya* with *Macrobrachium* than when alone, although not significantly higher.

Discussion

Rates of detrital processing varied along the elevational gradient as a function of the nature of the shrimp assemblage. We attribute the faster rate of leaf decay in controls at the high-elevation site primarily to shredding by *Xiphocaris* shrimp. Several lines of evidence support this. First, almost half the shrimps observed visiting leaf packs at the high-elevation site in the in situ experiment were *Xiphocaris*, and in the laboratory experiment, this species was able to break down leaves significantly faster than *Atya* or *Macrobrachium*. Furthermore, there was no difference in decay rates between laboratory treatments with *Atya* and *Macrobrachium* alone than in the no-shrimp control. Second, the only fish at this site is *S. plumieri*, which is algivorous (Erdman 1986) and was never observed entering the in situ control treatment. Third, insect shredders were rare and shredder biomass was not different between treatments. Insect biomass was significantly higher in controls at the high-elevation site; however, this was due to scraper–collector–gatherer mayflies (Ephemeroptera). Finally, a recent study conducted in a first-order stream near our high-elevation site also found that *Xiphocaris* significantly accelerated leaf litter decay (Crowl et al. 2001).

In addition to direct consumption, *Xiphocaris* may increase decay rates via facilitation of feeding by *Atya*. While atyid shrimps alone did not affect leaf decay rates in the laboratory experiment, rates were significantly faster than predicted when *Atya* and *Xiphocaris* were combined. When leaf material was partially shredded by *Xiphocaris*, the sweeping motion of atyid's chelipeds often removed pieces of leaf material from leaf packs (J.G. March, personal observation).

We attribute the lack of effect of *Xiphocaris* on leaf decay at the mid- and low-elevation sites to the greater abundance of *Macrobrachium*. Several lines of evidence from both the

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Table 2. Mean (±1 SE) number of shrimps observed in the control treatment during 2-min observation periods.

Site	Total shrimp[a]	*Atya*	*Macrobrachium*	*Xiphocaris*
High-elevation	0.62±0.17	0.12±0.05	0.02±0.02	0.13±0.06
Mid-elevation	0.80±0.23	0.00	0.17±0.05	0.63±0.22
Low-elevation	0.87±0.18	0.00	0.30±0.08	0.17±0.08

[a]Total shrimp observed in the control treatments includes unidentifiable shrimp.

Table 3. Mean (±1 SE) abundance (individuals·m^{-2}) and biomass (g wet weight·m^{-2}) of shrimps at each site obtained via electroshocking.

Site	Total shrimp	*Atya*	*Macrobrachium*	*Xiphocaris*
High-elevation				
Abundance	3.1±0.5	2.6±0.5	0.2±0.1	0.4±0.2
Biomass	3.09±1.09	2.39±1.12	0.38±0.31	0.31±0.13
Mid-elevation				
Abundance	6.1±1.0	0.3±0.2	4.1±0.9	1.8±0.7
Biomass	3.61±0.62	0.05±0.03	2.72±0.61	0.83±0.34
Low-elevation				
Abundance	6.9±0.9	0.1±0.3	6.2±0.7	0.4±0.3
Biomass	6.03±0.67	0.05±0.03	5.95±0.67	0.03±0.03

Fig. 1. Size–frequency distribution based on electroshocking of (*a*) *Atya*, (*b*) *Macrobrachium*, and (*c*) *Xiphocaris* shrimps at high- (hatched bars), mid- (open bars), and low-elevation sites (solid bars).

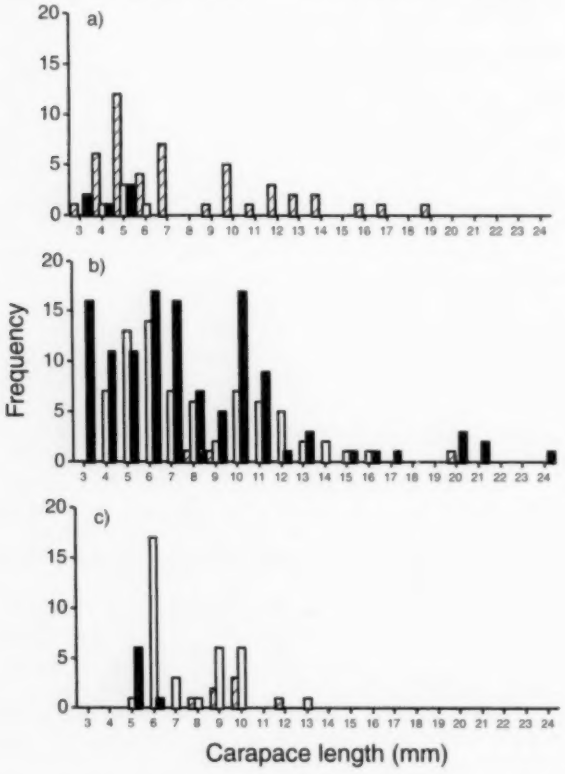
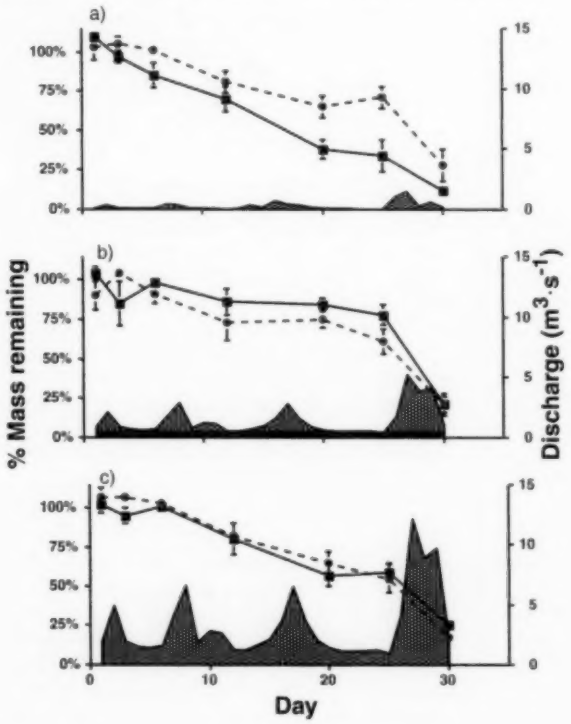


Fig. 2. Percentage of leaf pack mass remaining in control (squares, solid line) and shrimp-exclusion treatments (circles, broken line) and discharge (solid line, shading) at (*a*) high-, (*b*) mid-, and (*c*) low-elevation sites. Data are means ± 1 SE.



in situ and laboratory experiments support this hypothesis. First, *Macrobrachium* behaved aggressively towards other shrimp taxa in situ and in laboratory experiments. Second, in the laboratory experiment, *Xiphocaris* decomposed leaf litter faster than all other treatments. However, when *Xiphocaris* was combined with *Macrobrachium*, leaf decay rates were not different from the no-shrimp control. Third, decay in the

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Fig. 3. Insect biomass (ash-free dry mass) in control (squares, solid line) and shrimp-exclusion treatments (circles, broken line) at (*a*) high-, (*b*) mid-, and (*c*) low-elevation sites. Data are means ± 1 SE.

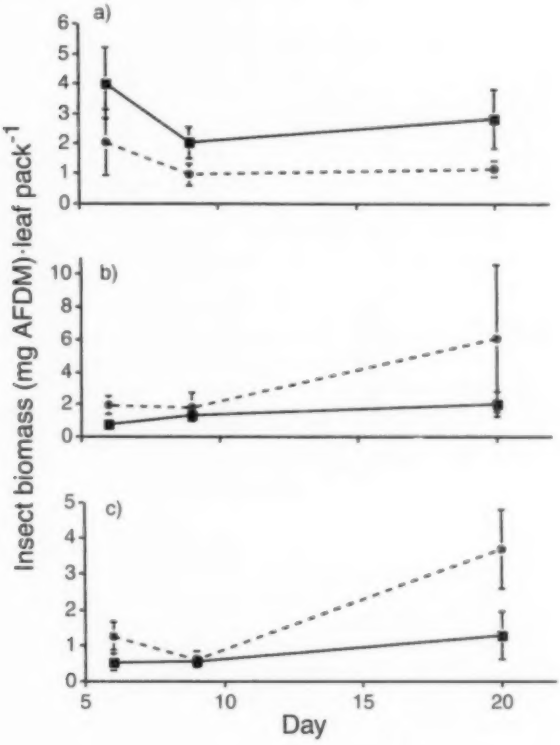
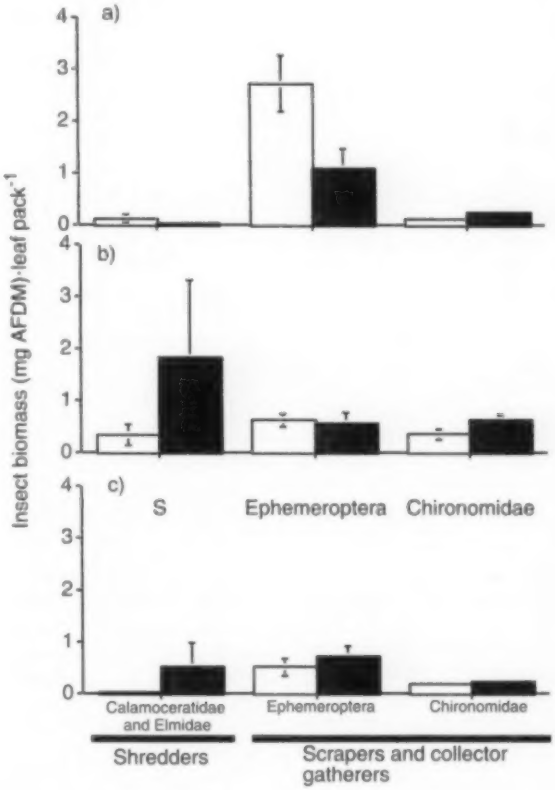


Fig. 4. Biomass (ash-free dry mass) of insect shredders and the two most common taxa, ephemeropterans and chironomids, in control (open bars) and shrimp-exclusion treatments (solid bars) over all dates at (*a*) high-, (*b*) mid-, and (*c*) low-elevation sites. Data are means ± 1 SE.



Xiphocaris and *Macrobrachium* treatment was significantly less than predicted based on decay rates in treatments with each taxon alone, suggesting a negative interaction between these two taxa. Fourth, mortality of *Xiphocaris* was significantly higher in the combined *Macrobrachium* and *Xiphocaris* treatment than in treatments where each taxon was alone. Other studies have also noted the aggressive behavior of *Macrobrachium* and its ability to cause other shrimp taxa to seek shelter (e.g., Crowl and Covich 1994). Our results strongly suggest that a shrimp predator (*Macrobrachium*) affected the activity of a shrimp shredder (*Xiphocaris*), thus resulting in a slower rate of leaf decay. This relationship has also been observed between insect predators and shredders in temperate streams (e.g., Oberndorfer et al. 1984).

Alternative explanations and discrepancies between our laboratory and in situ experiments must be considered in evaluating our results. *Xiphocaris* was observed to enter control treatments in greater numbers at mid- and low-elevation sites, yet rates of leaf decay were not different between treatments. There are two possible explanations for this discrepancy and they are not mutually exclusive. First, *Xiphocaris* is omnivorous and may shift its diet among sites because of differences in relative availability of food resources. Second, although *Xiphocaris* was observed more frequently in controls at the lower sites relative to the high-elevation site, they may have been moving out of controls as fast as they were moving in. We recorded how many shrimps were present in controls but did not measure time spent feeding. Thus, higher numbers of *Xiphocaris* at the mid- and low-elevation sites may have given rise to higher turnover of shrimps in controls, but high numbers of *Macrobrachium* disrupted their feeding, causing lower feeding pressure relative to the high-elevation site. The fact that we observed greater direct negative effects of *Macrobrachium* on *Xiphocaris* in the laboratory versus in situ experiment is not surprising. Typically, strong negative effects of predators on prey are found in cages with small mesh size or aquaria that are impermeable and allow no prey immigration and emigration (Cooper et al. 1990). However, studies that allow natural exchange of prey often find no effect of predators on prey abundance (e.g., Malmqvist 1993).

Many factors affect leaf decay, and the relative importance of each factor may change within and among streams (e.g., Minshall et al. 1983). Changes in abiotic factors among our three sites could potentially contribute to differences in leaf decay rates. Both temperature and water velocity increased in the downstream direction. However, we found no differences in leaf decay rates in shrimp-exclusion treatments

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Fig. 5. Differences in leaf decomposition among treatments in the laboratory aquarium experiment. Different lowercase letters above each treatment show statistical differences ($P < 0.05$). Taxa in each treatment: A, *Atya*; X, *Xiphocaris*; M, *Macrobrachium*. Data are means ± 1 SE.

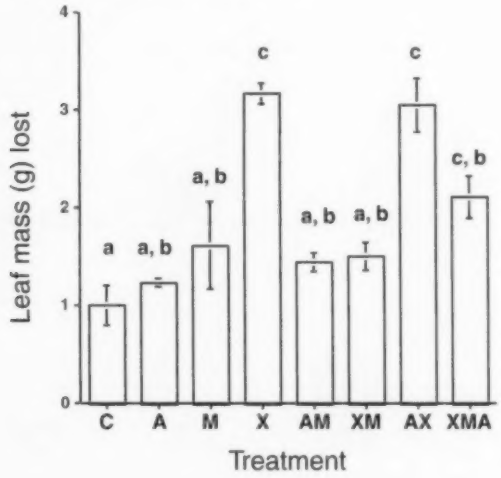
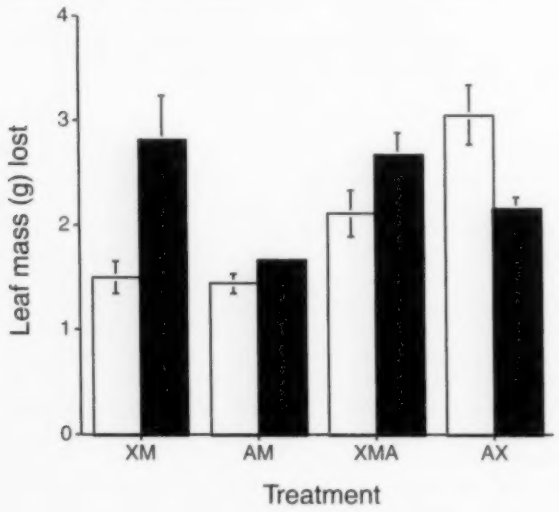


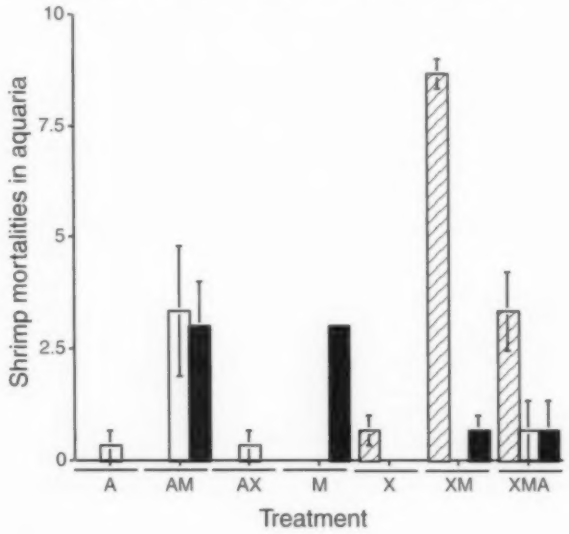
Fig. 6. Differences between observed (open bars) and predicted leaf decomposition (solid bars) in multiple-taxa treatments. Predicted values greater than observed indicate a negative interaction between taxa (interference competition–predation). Predicted values less than observed indicate a positive interaction between taxa (facilitation). Taxa in each treatment: A, *Atya*; X, *Xiphocaris*; M, *Macrobrachium*.



among sites. In the absence of shrimps, therefore, differences in abiotic factors between sites had no effect on leaf decay.

In our study, insect shredders were not affected by shrimps and did not appear to play an important role in leaf decay. This finding contrasts with studies in temperate headwater streams where insect shredders play a major role in organic matter processing (Wallace and Webster 1996). However, studies in other tropical streams also suggest that insect shredders play less of a role in leaf decay compared with temperate streams (Irons et al. 1994; Ramirez and Pringle 1998; but see Benstead 1996).

Fig. 7. Mortality of *Xiphocaris* (hatched bars), *Atya* (open bars), and *Macrobrachium* (solid bars) in different treatments. Taxa in each treatment: A, *Atya*; X, *Xiphocaris*; M, *Macrobrachium*.



Shrimps positively affected mayfly biomass. However, this effect of shrimps on mayflies was only observed at the high-elevation site. Mobile mayflies may be able to avoid predation by shrimps while benefitting from sediment removal (mostly by atyids), which increases access to underlying algae (Pringle et al. 1993) or biofilm on leaves. The absence of a positive response of ephemeropterans to shrimps at the mid- and low-elevation sites may have been due to the absence of large atyids and a consequent lack of any resource enhancement effect at these two sites.

In contrast with mobile mayflies, a greater biomass of sessile chironomids was found in the absence of shrimps at all sites. Less mobile chironomids may be more vulnerable to predation by shrimps. Two other studies conducted in tributaries near the high-elevation site have found similar results. Pringle et al. (1993) found a reduction of sessile chironomids and an increase in mobile mayflies in the presence of *Atya* and *Xiphocaris* on clay tiles at high-elevation sites. Likewise, Buzby (1998) found more mobile insect taxa in a stream dominated by shrimps and more sessile taxa in shrimp-poor streams.

Understanding effects of shrimps along stream continua is of increasing importance due to anthropogenic disturbances in rivers of Puerto Rico. Holmquist et al. (1998) demonstrated that high dams act as barriers that restrict shrimps to reaches below dams. Furthermore, Benstead et al. (1999) showed that water withdrawals from low-elevation reaches result in significant mortality of shrimp larvae during their migratory drift to estuarine larval habitat. Reduction or elimination of freshwater shrimps in headwaters could result in

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dramatic changes in ecosystem processes such as leaf decay as well as changes in the quality and quantity of benthic organic matter and algal and invertebrate community composition (Pringle et al. 1999).

In summary, our in situ results showed that shrimps can significantly increase rate of leaf decay and increase or decrease insect biomass; however, these effects were dependent on location along the elevational gradient. The laboratory aquarium experiment showed that *Xiphocaris* was the most important shredder within the shrimp assemblage and suggested that interference competition and (or) predation by *Macrobrachium* can result in decreased litter processing by *Xiphocaris*. This study demonstrates that shrimp assemblages can have dramatic effects both on an important ecosystem process (leaf decay) and on community structure (benthic insect biomass). Moreover, our study illustrates the importance of examining the roles of species assemblages at multiple sites. Had we conducted our study at only one site, our interpretation of shrimp effects on leaf decay and insects would have been limited. Examining the roles of species assemblages over a variety of sites provides a more thorough understanding of their roles in ecosystems as well as insights into the potential consequences of human-induced shifts in species distributions.

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A regional paleolimnological assessment of the impact of clear-cutting on lakes from the west coast of Vancouver Island, British Columbia

Kathleen Laird, Brian Cumming, and Rick Nordin

Abstract: The impact of forest harvesting on lakes within the temperate rainforest on the west coast of Vancouver Island was examined in a paleolimnological study of four lakes that had 35–92% of their watersheds progressively clear-cut over a period of 15–30 years (impact lakes) and four lakes that had experienced little or no known anthropogenic disturbance in their watersheds (reference lakes). Changes in diatom species composition and percent organic matter in the ^{210}Pb-dated sediment cores were compared over the last 100 years in each of the impact lakes before and after the onset of forest harvesting, which began in 1950, and before and after 1950 in the four reference lakes. Only one impact lake showed significant changes in percent organic matter. Significant changes ($p < 0.05$) in species composition following forest harvesting were detected in all four impact lakes and in one of the four reference lakes. However, the changes in diatom species composition following clear-cutting in the impact lakes were small, with changes in the relative abundance of the most common species being maximally 20%, but more typically 3–10%.

Résumé : L'impact de la coupe de la forêt avoisinante sur les lacs dans la forêt pluvieuse tempérée de la côte occidentale de l'île de Vancouver a pu être déterminé par une étude paléolimnologique de quatre lacs dont 35–92% du bassin versant a été progressivement soumis à la coupe à blanc sur une période de 15–30 ans (lacs affectés) et de quatre autres lacs dont les bassins versants ont subi, à notre connaissance, peu ou pas de perturbations anthropiques (lacs témoins). Nous avons examiné les changements de la composition spécifique des diatomées et du contenu en pour cent de matière organique de carottes de sédiments datés au ^{210}Pb au cours des 100 dernières années, soit dans chacun des lacs affectés avant et après la coupe de la forêt qui a débuté en 1950 et dans les quatre lacs témoins avant et après 1950. Seul un des lacs affectés avait subi un changement significatif du pourcentage de matière organique. Des modifications significatives ($p < 0.05$) de la composition spécifique des diatomées après la coupe de la forêt ont été décelées dans les quatre lacs affectés et un lac témoin; cependant, ces changements étaient faibles, avec une variation des densités relatives des espèces les plus communes d'au plus 20%, et plus communément de 3–10%.

[Traduit par la Rédaction]

Introduction

In British Columbia (B.C.), over 90% of the forests are managed under either Timber Supply Areas or Tree Farm Licenses (TFLs), of which the latter are given to individual companies (Pedersen 1995). The TFLs, which account for 8% of the province, contribute approximately 20% of the annual harvest volume (Pedersen 1995) and are concentrated along the coastal regions of Vancouver Island, home to a large portion of the world's remaining temperate rainforests. In the 1980s and 1990s, provincial forest managers started to view the forest as more than a supply of wood and began to manage with a concern for wilderness, biodiversity (Kimmins 1995), and impacts on aquatic systems (Hartman and Scrivener 1990). In 1995, the Forest Practices Code of British Columbia was established, to promote the conservation of biological diversity through management of the forests, based on ecological units, watersheds, or groups of watersheds (Fenger 1996).

Management of the forested region within a watershed needs to take into account the meteorological, geological, and biological processes that link the terrestrial and aquatic ecosystems. For instance, clear-cutting has been shown in numerous studies to enhance flow, turbidity, concentration of dissolved ions, and temperature in streams (see review in Keenan and Kimmins 1993). The impact of forest harvesting on lakes has been grossly understudied in comparison with research on streams. Furthermore, most studies on the impact of forestry activities on lakes have typically been concerned with short-term dynamics (e.g., Rask et al. 1998). Even in a recent special issue on watershed perturbations (Carignan and Steedman 2000), the majority of studies are only 3 years in duration and few contain any preharvest limnological data.

Paleolimnological techniques can provide a means of assessing impacts associated with forestry activities over a longer time frame, because the sediment record provides an archive of past changes of many biological, chemical, and physical indicators of environmental change. For example, in

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northwestern Ontario, lake sediments were used to assess the impacts of logging, fire, and climate on impact and reference lakes over the past 30 years (Paterson et al. 1998).

The purpose of this study was to examine the impact of forest-harvesting activities on lakes within the temperate rainforest on the west coast of Vancouver Island. Changes in the sediment organic matter and diatom assemblages preserved in the lake sediments over the last 100 years were compared within four lakes before and after the onset of forest harvesting (impact lakes) and in four lakes in the same region that had experienced little or no known watershed disturbance (reference lakes). Diatom analysis was the main focus of this study, because they are one of the most widely used and sensitive indicators of past changes within lakes (e.g., Stoermer and Smol 1999). This study was designed to detect changes associated with continuous logging practices that occurred over many decades, and provides an assessment of natural variability in the reference lakes and in the impact lakes prior to clear-cutting. Furthermore, changes in the impact lakes (post logging), in comparison with the reference lakes, can be used to determine if the changes in the impact lakes are predominantly attributable to logging activities or potentially related to more regional changes such as climate. A similar study was carried out in the central interior of British Columbia and is presented elsewhere (Laird and Cumming 2001, this issue).

Materials and methods

Study area, sites, and study design

The lakes examined in this study are located on the west coast of Vancouver Island near the towns of Ucluelet and Bamfield on Barkley Sound (Fig. 1). This region lies within the Coastal Western Hemlock biogeoclimatic zone and is dominated by western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and western red cedar (*Thuja plicata* Donn). The area receives very high levels of precipitation (250–500 cm·year^{-1}), falling primarily as rain from October to March, with increasing annual precipitation as one moves inland (Valentine et al. 1978). The dominant soils are acidic ferro-humic podzols, which are low in base cations (Valentine et al. 1978) and nutrients (Clayoquot Sound Scientific Panel 1995), and the landscape is characterized by gentle to extreme slopes.

The lakes in this study were selected from government databases (e.g., Ministry of Forests files, Ministry of Environment fisheries inventory files, maps, and air photos), based on the following criteria: (*i*) the presence of both reference and logged lakes within an area of similar precipitation, geology, and vegetation; (*ii*) the absence of large-scale natural disturbances, such as fire, in the last 100 years; and (*iii*) an attempt to select impact and reference lakes within a similar range of physical (e.g., lake area, watershed area, depth, simple bathymetry) and chemical (e.g., pH, conductivity, nutrients) characteristics (Table 1). In total, 12 lakes were cored. However, based on the results of the ^{210}Pb profiles, detailed logging histories of the basins, and concentration and preservation of diatom assemblages in the cores, only eight lakes were analysed in detail. Owing to the difficulty of finding lakes that had experienced no forestry activities in the past 100 years, our reference lakes are defined as having no logging prior to 1980, since this date incorporates most of the prelogging interval in these watersheds. Samples after the onset of logging in the reference lakes were deleted from any analyses. Clear-cutting began in most of the impact lakes in ca. 1950. One of the originally selected impact lakes that experienced logging at this time had low diatom abundances and was not included in the study. In addition, Black Lake, initially selected as a reference lake, had an earlier onset of logging, with associated road building, than originally realized and was therefore analyzed as an impact lake. Thus, three lakes with cutting primarily between 1950 and 1985 (Maggie, Pachena, and Sugsaw lakes) and one lake with cutting between 1970 and 1990 (Black Lake) were included in the analyses.

Field sampling

The lakes were sampled from a Beaver float plane, owing to either no road access or difficult access by logging roads. Two cores were retrieved from the deep basin of each lake, using a modified K-B gravity corer (Glew 1989) equipped with a 60 cm long core tube (internal diameter ~7.6 cm). Approximately 4 kg of extra weight was attached to the approximately 9 kg corer, to provide deeper penetration of the core tubes into the sediments. The cores ranged in length from 35 to 45 cm and were secured upright in padded buckets during transport by plane.

The cores were sectioned vertically into polyethylene whirlpack bags within 24–36 h of core retrieval. Based on estimations of sedimentation rates, the assumption was made that background ^{210}Pb activity would occur by 20 cm in most systems. Thus, each core was sectioned every 0.25 cm for the top 20 cm, and every 0.5 cm for the rest of the core, with the exception of the core from Maggie Lake, which was sectioned every 0.25 cm for the top 30 cm and then every 0.5 cm. Maggie Lake was sectioned in this manner, because initial core evidence suggested that sedimentation was possibly more rapid in this system. Core samples from each lake were placed in large plastic bags, stored in a cooler on ice, and shipped to Queen's University, Kingston, Ontario, within 48 h of sectioning, where they were stored at 4°C.

Water samples were collected approximately 0.5 m below the water surface of each lake, stored on ice, and shipped to the Pacific Environmental Science Center (Canadian Federal Government Laboratory) in North Vancouver, B.C., within 24 h of collection. Chemical analyses followed standard procedures outlined by the American Public Health Association (1989). Water samples were analyzed for total phosphorus, total nitrogen, nitrate, nitrite, all major anions and cations, dissolved metals (Al, B, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Bd, Z, Sr), dissolved inorganic carbon, dissolved organic carbon, alkalinity, and silica. Total phosphorus and nitrogen were estimated from unfiltered samples; all other analyses were based on water samples filtered in the field through a 0.45-μm cellulose acetate filter into acid-washed bottles. Estimates of chlorophyll *a* were obtained using standard methods based on a 1000-mL sample that was filtered through a 0.45-μm cellulose acetate filter.

^{210}Pb dating, percent organic matter, and diatom preparation

From 18 to 22 subsamples of wet sediment from one core from each lake were weighed in a crucible, oven-dried (24 h at 105°C), and reweighed, to determine the percent water and dry weight of the sediment. Samples were ground to a fine dust in the crucible using a pestle and redried overnight at 105°C. Dried sediment was shipped to MYCORE Ltd., Deep River, Ontario, for ^{210}Pb analysis. After the ^{210}Pb results were received for the first cores, the second core from four of the study lakes was subsampled for ^{210}Pb as above, to determine reproducibility of the ^{210}Pb profiles for cores with exponential decay profiles (Black and Pachena lakes), as well as for those with less characteristic ^{210}Pb profiles (Maggie and Toquart lakes).

^{210}Pb activity for each sample was estimated by alpha spectroscopy, using a ^{209}Po tracer of known activity. Unsupported ^{210}Pb was calculated by subtracting supported ^{210}Pb (the baseline activity determined from bottom samples of the core using the guidelines in Binford (1990)) from the total activity at each level. The sedi-

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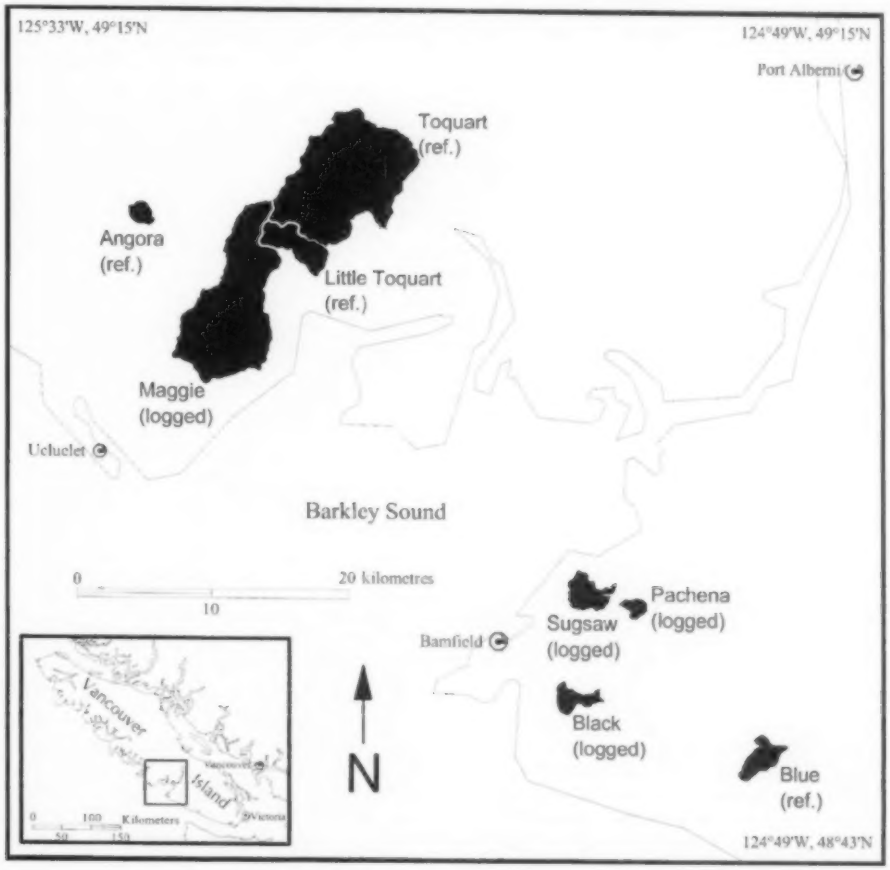
Fig. 1. Location of the watersheds for the reference (ref.) and impact (logged) lakes. Approximate latitude and longitude are given in the corners of the diagram. Modified from Spicer (1999).



ment chronology and sedimentation rates were calculated from the estimates of ^{210}Pb activities and estimates of cumulative dry mass (Binford 1990), using the constant rate of supply (CRS) model (Appleby and Oldfield 1978). Chronology for all cores is based on linear interpolation between dated intervals.

The percent organic matter in each core since ca. 1900 was determined in consecutive 0.25-cm intervals (ca. 2- to 3-year resolution) using standard loss-on-ignition methods (Dean 1974). Briefly, a known quantity of dried sediment was heated to 550°C for 2 h in a NEY® model 2-21350 muffle furnace with digital temperature control. The difference between the original weight of the dried sediment and the weight of sediment remaining after ignition was used to estimate the percent organic matter in each sediment sample.

Slides for diatom analysis were prepared using standard techniques (e.g., Wilson et al. 1996). For each sample, at least 400 diatom valves were enumerated along transects on a Leica DMRB microscope under oil immersion at 1000× magnification, using an objective with a numerical aperture of 1.3. Diatom species identification follows references outlined in Wilson et al. (1996).

Statistical analyses

Analysis of similarities (ANOSIM), a nonparametric multivariate statistical test (Clark and Warwick 1994), was used to test the null hypothesis that there was no difference in diatom species composition before and after the onset of forest harvesting in the impact lakes or before and after 1950 in the reference lakes. The approximate date of onset of logging for three of the four impact lakes was 1950. Because the reference lakes were logged after 1980, intervals after the onset of logging in the reference lakes were excluded from the ANOSIM analyses. For example, in Blue Lake, logging started in 1992, thus the intervals included in the "after" group consisted of intervals between 1951 and 1991. Prior to running ANOSIM for Maggie, Toquart, and Sugsaw lakes, three to five samples were deleted, to provide approximately equal temporal resolution between intervals to ensure that results were not biased owing to different resolutions before and after 1950.

ANOSIM uses a rank similarity or dissimilarity matrix to calculate within group and across-group differences (Clark and Warwick 1994). A Bray–Curtis similarity coefficient with no species transformations was used as the similarity coefficient in our analyses. Rare taxa (present at less than 2% relative abundance in two or fewer samples) were grouped into diatom genera or other morphological categories (e.g., rare *Navicula*, rare centrics, rare araphes). ANOSIM tests were calculated using the statistical package PRIMER 4.0 (Plymouth Marine Laboratory 1996). The outcome of an ANOSIM comparison is termed an R statistic, the value of which may vary between –1 and 1. An R statistic near 0 indicates that the null hypothesis of no differences between the two groups of samples is likely true (i.e., the within- and between-group differences are similar), whereas an R of 1 indicates that samples are always more similar to each other when compared within each group

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Table 1. Summary of the physical and chemical characteristics of the lakes studied.

	Reference lakes				Impact lakes			
	Angora	Blue	Little Toquart	Toquart	Black	Maggie	Pachena	Sugsaw
Lake area (km^2)	0.31	0.47	0.55	1.15	0.64	2.49	0.54	0.61
Watershed area (km^2)	2.1	5.3	9.9	66.8	4.6	54.3	1.9	5.5
Watershed cut (km^2)	—	—	—	—	1.27	32	1.51	5.52
Watershed cut (%)	0	0	0	0	35.1	59	79.1	92
Watershed/lake area	6.8	11.3	18	58.1	5.6	21.8	3.5	9.8
Maximum depth (m)	46.5	11	16	37.5	27.2	45	25.8	31
Coring depth (m)	36	11	14	33	26	35	23	19
Volume (10^6 m^3)	5.9	na	na	24	14	na	6.3	6
Mean watershed slope	0.35	0.05	0.14	0.12	0.11	0.06	0.23	0.14
pH	7.75	6.96	6.54	6.66	7.14	6.65	7.23	7.15
Specific conductivity ($\mu S \cdot cm^{-1}$)	42.8	24.3	22.5	28.6	30.2	39.5	27.3	29.4
Alkalinity ($mg \cdot L^{-1}$)	13	4	8.2	13.9	9.5	14.6	7.7	9.1
Total phosphorus ($\mu g \cdot L^{-1}$)	4	5	3	3	2	19	12	3
Total nitrogen ($\mu g \cdot L^{-1}$)	90	170	130	110	160	100	120	190
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	0.5	1.9	0.5	0.5	0.7	1.1	0.5	0.8
Silica ($mg \cdot L^{-1}$)	1.48	0.47	0.86	1.32	1.1	1.2	0.98	1.2
Calcium ($mg \cdot L^{-1}$)	5.3	1.1	1.3	3.7	1.9	4.4	1.9	1.9
Sodium ($mg \cdot L^{-1}$)	2.2	2.4	1.8	1.3	2.9	2	2.1	2.5
Chloride ($mg \cdot L^{-1}$)	3.1	3.8	2.7	1.7	4.5	2.8	3.3	4
Dissolved inorganic carbon ($mg \cdot L^{-1}$)	3.2	0.7	0.6	2.8	1.3	3.2	1.5	1.4
Dissolved organic carbon ($mg \cdot L^{-1}$)	3.6	7.6	6.3	2.5	5.8	3.7	3.4	5.1

Note: Chemical variables are based on one sample taken in August 1996. For reference lakes, watershed cut is before 1980 and, for logged lakes, is total cut. Mean watershed slope is calculated by H/L, where H is the difference between the highest and lowest point in the basin and L is the horizontal distance along the longest dimension of the basin parallel to the principal drainage line (Schumm 1956).

than when compared across groups. The significance of the R statistic was evaluated using resampling procedures, in which replicates were rearranged into all possible combinations; an R statistic is calculated during each rearrangement. The resulting distribution of R statistics was then used to assess the probability that the R statistic based on the original groupings could be the result of chance. The significance level of $p \leq 0.05$ was chosen a priori. Following the ANOSIM analysis, the program SIMPER in the statistical package PRIMER 4.0 (Plymouth Marine Laboratory 1996) was used to calculate the contribution of each species to the average dissimilarity between the two groups.

Profiles of organic-matter changes in the impact lakes were split a priori into two groups according to the ^{210}Pb estimated dates, those intervals that occurred before the onset of logging and those that occurred after logging. For the reference lakes, the samples were split into two groups, before and after 1950, with intervals after the onset of logging being deleted before the analysis (see explanation above). The two groups were tested for significant difference using a t test, in which the significance was evaluated using the program Resampling Stats® v. 3.16. Samples equal in size to the original two groups were chosen at random from the entire sample set (without replacement) 1000 times and t tests were performed on these to generate a distribution of t values that could appear by chance for each dataset. If the original t value was in the extreme 5% ($p < 0.05$) of this distribution, then the null hypothesis that there was no difference in mean organic matter between the two groups was rejected.

Principal components analysis (PCA) and canonical variates analysis (CVA) were carried out with the computer program CANOCO 4.0 (ter Braak and Šmilauer 1998). PCA was carried out on physical characteristics of the watersheds (maximum depth, lake area, watershed area, watershed/lake area, total stream length, and slope) using a correlation matrix, to explore whether any of the lakes (reference and impact) were different from the others based on these physical characteristics. All variables except depth, were log-transformed to obtain a more normal distribution of the values prior to analysis. CVA analysis was used to determine if the difference between the two groups of lakes, those that showed a significant response in the ANOSIM analysis and those that did not, could be explained by any of the measured physical characteristics or by a linear combination of these characteristics.

Results

^{210}Pb profiles

The ^{210}Pb profiles from the reference and impact lakes all show decreases in activity with depth and all indicate that the cores were of sufficient length to reach supported levels of ^{210}Pb activity (Fig. 2). Log of total ^{210}Pb versus cumulative dry weight was plotted to indicate the values from which supported ^{210}Pb were calculated (Fig. 2). Plots of log unsupported ^{210}Pb show similar trends to those presented and, for most cores, are indistinguishable post 1900 A.D. from log of total ^{210}Pb, owing to the small and constant amount of supported ^{210}Pb. The ^{210}Pb profiles do show that all lakes experienced either some changes in their sedimentation rates or changes in sediment mixing over the time frame represented by these cores (i.e., changes in the slope of log ^{210}Pb versus cumulative dry weight in the region of unsupported ^{210}Pb activity). During the period of interest in this study (ca. 1900 to present), changes in sedimentation and (or) mixing regimes were minimal in two of the four reference lakes (e.g., Figs. 2*a* and 2*c*). In the other two reference lakes (Figs. 2*b* and 2*d*), the ^{210}Pb profiles suggest that there were some changes in the sedimentation rates or sedi-

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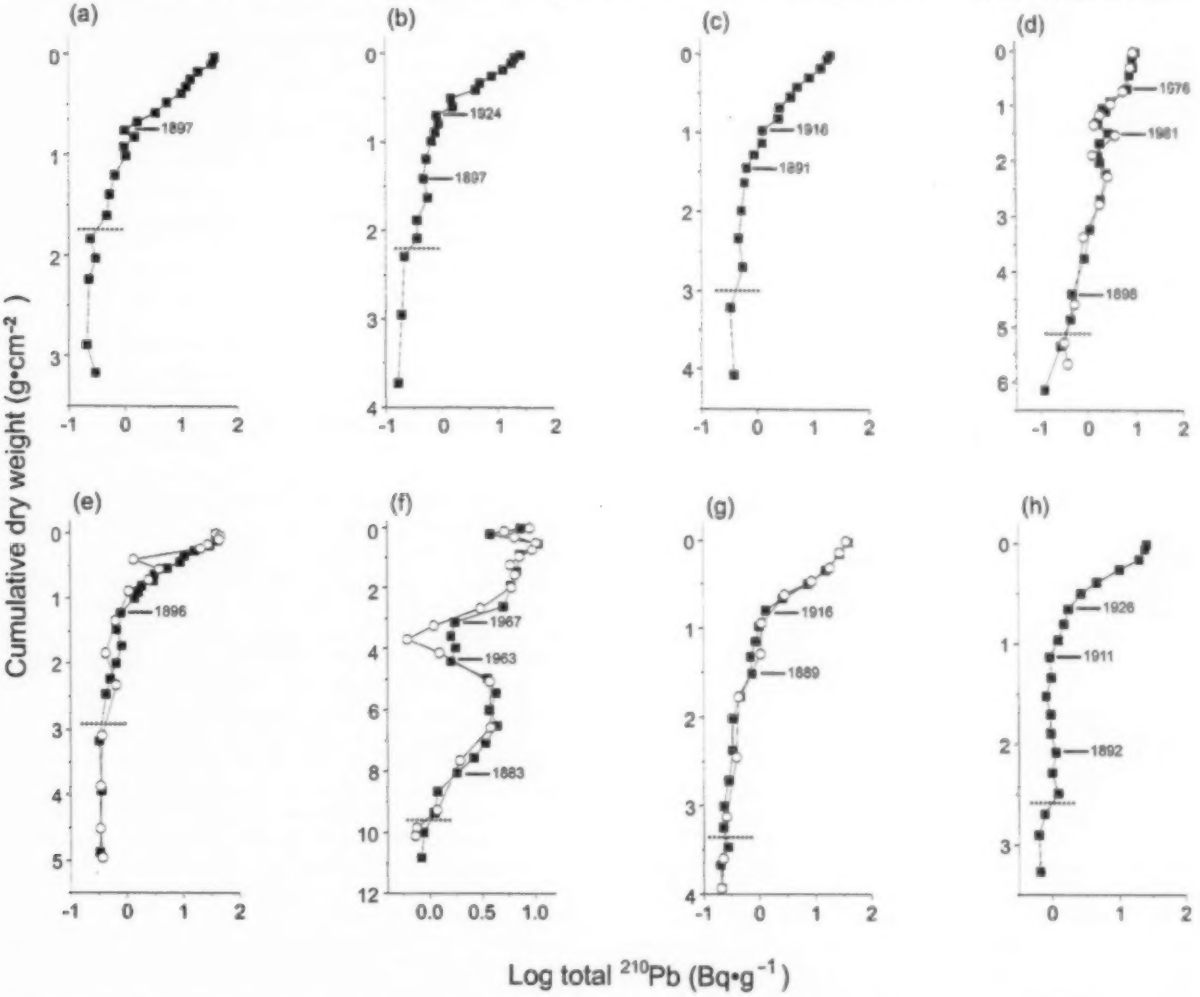
Fig. 2. Log of total ^{210}Pb activity versus cumulative dry weight for reference (*a–d*) and impact (logged, *e–h*) lakes: reference lakes: (*a*) Angora, (*b*) Blue, (*c*) Little Toquart, and (*d*) Toquart; logged lakes: (*e*) Black, (*f*) Maggie, (*g*) Pachena, and (*h*) Sugsaw. Core analyses were completed for profiles shown using solid squares. In four lakes, the second core retrieved from the lake was also dated (open circles). Approximate dates, estimated from the constant rate of supply (CRS) model, are given for points of change in slope and the beginning date of focus of this study (1890–1900). Supported ^{210}Pb is indicated as samples below the dotted horizontal line.



ment mixing. For example, in Blue Lake (Fig. 2*b*), the decrease in the slope of activity versus cumulative dry weight between ~0 and 0.5 g·cm^{-2} could be interpreted as a decrease in sedimentation rate or as a decrease in the average rate of sediment mixing. Similarly, the region of relatively constant activity between 1 and 2.5 g·cm^{-2} in Toquart Lake (Fig. 2*d*) could be interpreted as a region of increased sediment deposition or of increased mixing.

Three of the four impact lakes show little evidence of changes in sedimentation rate or mixing regime during the time frame of interest of this study (Figs. 2*e*, 2*g*, and 2*h*). This is particularly true for Black Lake (Fig. 2*e*), whereas Pachena Lake (Fig. 2*g*) and Sugsaw Lake (Fig. 2*h*) indicate small changes in slope around 1916 and 1926, respectively, which may indicate either a decrease in sedimentation rate or a decrease in the average rate of mixing. The fourth lake shows major departures from an exponential decay curve (Fig. 2*f*), suggesting that large changes in sedimentation and (or) mixing occurred in this lake. The CRS model dates the large decline in ^{210}Pb activity in Maggie Lake at between ca. 1963 and 1967 (Fig. 2*f*). This portion of the sediment core was associated with large amounts of organic debris, including wood and needles. A smaller event appears to have occurred towards the top of this core (Fig. 2*f*).

Reproducibility of the ^{210}Pb profiles from the replicate cores in the deep basin was excellent for all profiles (Figs. 2*d–2g*). Even though some ^{210}Pb profiles suggest that either changes in sedimentation rate or mixing occurred, this does not hamper our conclusions, because this study was designed to detect changes associated with continuous logging practices occurring over many decades, and our conclusions are drawn from all lakes, which show similar trends in spe-

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cies changes (see below). Overall, the ^{210}Pb results obtained from the reference and impact lakes suggest that reliable chronologies can be estimated from these cores.

Organic matter changes and resampling results

Only one logged lake (Pachena) showed a significant difference in percent organic matter before and after the onset of clear-cutting (Table 2). Coincident with the onset of logging in the watershed of Pachena Lake, there was a gradual decrease in percent organic matter from approximately 45% to approximately 30% by 1990 (Fig. 3*g*). After 1990, organic matter began to increase.

Although Maggie Lake did not show a significant difference in organic matter before and after the onset of logging, a distinct but short-lived peak in organic matter was found that corresponded approximately to the onset of logging (Fig. 3*f*). This distinct peak in organic matter is composed of a 5- to 6-cm layer of woody debris (chunks of wood, twigs, needles) that is estimated (from the two ^{210}Pb-dated cores) to have been deposited within a short time span (1–4 years) and is associated with large decreases in ^{210}Pb activity between 1963 and 1967 (Fig. 2*f*). In 1962, the same time as the onset of logging and road building, Hurricane Freda hit the region, bringing in torrents of rain that may have started debris slides on an already disturbed landscape. Concurrent with the logging and Hurricane Freda, an open pit iron mine upstream from Maggie Lake operated from 1962 to 1968 (Spicer 1999). Consequently, it is impossible to untangle the specific contributions of these various disturbances to the observed changes in sedimentation and organic matter. A later peak in organic matter in the 1990s at Maggie Lake may, in part, be associated with a large January storm in 1992, during which nearly 75 cm of rain fell in the Barkley Sound area (Spicer 1999). The increased flow of water on a disturbed landscape may have resulted in debris slides, as described earlier. Disregarding these peaks in organic matter, there is a general decrease in organic matter from approximately 20% prior to logging to 11–15% from 1970 to 1985 that may be associated with increased erosion of inorganic mineral inputs from the watershed.

In Toquart Lake (reference lake), a large peak in organic matter centered around 1952 was found, with another smaller peak in the 1960s (Fig. 3*d*). No known anthropogenic disturbances occurred in this watershed during this time interval (John Deniseger, Ministry of the Environment, Nanaimo, B.C., personal communication), thus the cause of the increase in organic mattter is unknown. Estimated dates suggest that the organic matter increased over a fairly long time span and, thus, it seems unlikely that the peak is associated with an erosional event. Furthermore, there is no evidence of terrestrial debris in the core and no decline in ^{210}Pb activity associated with the peak in organic matter, as was observed in Maggie Lake. There was a decline in ^{210}Pb activity between 5 amd 6 cm (estimated date of 1967–1970); however, this decline corresponds to the very end of the peak in organic matter. In addition, the increase in organic matter corresponds to increases in *Aulacoseira* spp. and araphid diatom taxa between 1950 and 1970, indicating the significance of this event in the history of this lake (Fig. 4*d*); however, the cause of this change is uncertain.

Diatom species changes and ANOSIM results

The diatom flora was very diverse in all the lakes (Figs. 4 and 5), with the exception of Angora Lake (Fig. 4*a*), which had only 20 taxa. All other lakes had more than 100 diatom taxa, with up to 170–190 taxa in Blue (Fig. 4*b*) and Maggie (Fig. 5*b*) lakes. Dominant taxa, which together typically comprise greater than 50% of the diatom assemblages, include the planktonic centric species *Cyclotella stelligera* (Cleve and Grunow in Cleve) Van Heurck, *Cyclotella pseudostelligera* Hustedt, *Cyclotella glomerata* Bachmann, *Aulacoseira distans* (Ehrenberg) Kützing, and *Aulacoseira distans* var. *tenella* (Nygaard) Florin. Blue and Little Toquart lakes (Figs. 4*b* and 4*c*), the two shallowest lakes, are exceptions, with the most abundant taxa being the benthic taxa *Brachysira brebissonii* Ross and *Frustulia rhomboides* (Ehrenberg) De Toni; these comprise approximately 20 and 10–15% of the assemblages, respectively. The periphytic taxon *Achnanthes minutissima* Kützing was abundant in Toquart (Fig. 4*d*) and Maggie (Fig. 5*b*) lakes, typically comprising 25–30% of the assemblage in Toquart Lake and 10–20% in Maggie Lake. The genera *Navicula* Bory and *Eunotia* Ehrenberg were extremely diverse in the study lakes, with the mean number of taxa across lakes being 26 and 29, respectively, but any specific taxon was rare. Other diverse genera across all lakes include *Achnanthes* Bory and *Cymbella* Agardh, whereas the genus *Gomphonema* Agardh was only diverse and of relative importance in Maggie Lake.

Sediment intervals in each lake were divided a priori into two groups according to the ^{210}Pb estimated dates: before and after forest harvesting for the impact lakes and before and after 1950 for the reference lakes. The difference in species assemblages between the two groups within each lake was tested using ANOSIM. The null hypothesis of no difference between diatom assemblages in the two groups could not be accepted in five of the eight lakes (Table 3). All the impact lakes (Black, Maggie, Pachena, and Sugsaw) and one reference lake (Little Toquart) showed a significant difference in species assemblage between the two groups. However, in only three of the lakes, two impact lakes (Black and Pachena) and one reference lake (Little Toquart), are these differences associated with a high R statistic (Table 3). Although the variations in diatom species abundances in the lakes that showed significant differences between the two time periods are often quite small, with changes in the relative abundance of the most common species being maximally 20% (but more typically 3–10% (Figs. 4 and 5)), the variations in diatom community composition are, as expected, more distinct in those lakes that had a high R value. In Pachena Lake (Fig. 5*c*), there was a distinct increase in the abundance of *C. pseudostelligera* and *C. glomerata*, whereas taxa such as *A. distans* and *A. distans* var. *tenella* decreased in abundance; however there appears to be a lag in the response of *A. distans* with respect to the onset of forest-harvesting activities. In Black Lake (Fig. 5*a*), the change in the two taxa that contributed the most to the average dissimilarity between the before and after groups (as determined using SIMPER), *A. distans* var. *tenella* and *C. pseudostelligera*, is not strongly associate with the timing of the onset of logging, as in Pachena Lake. However, other taxa with relatively high contributions, such as *C. stelligera* and *A. distans*, increase

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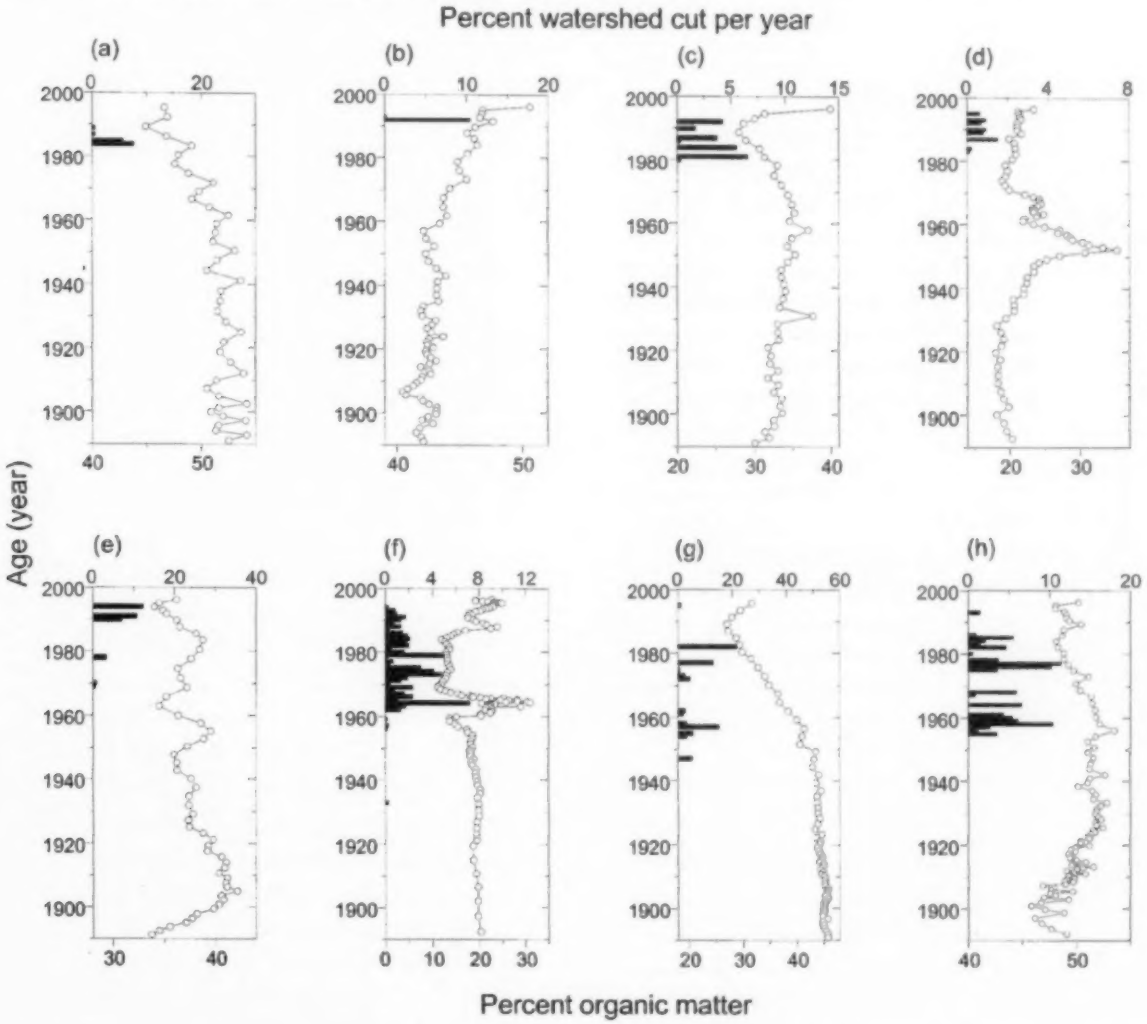
Table 2. Mean percent organic matter (OM) of samples before and after 1950, the date of the onset of logging in the impact lakes.

	Reference lakes				Impact lakes			
	Angora	Blue	Little Toquart	Toquart	Black	Maggie	Pachena	Sugsaw
Mean percent OM before	52.2±1.2	42.5±0.7	33.1±1.4	20.3±2.0	38.0±2.2	19.4±0.6	44.6±0.8	49.9±2.0
	(20)	(29)	(24)	(29)	(32)	(28)	(33)	(41)
Mean percent OM after	50.3±1.7	44.3±1.5	34.5±1.2	24.8±4.3	36.5±1.5	17.6±4.6	33.6±5.0	50.1±1.5
	(13)	(17)	(11)	(32)	(14)	(41)	(19)	(28)
t	−3.79	5.69	3.05	5.09	−2.37	−2.00	−12.48	0.30
p	0.19	0.15	0.26	0.34	0.50	0.73	0.005**	0.96

Note: Values are mean ± 1 SD, with sample size (n) in parentheses. The t and p are given for testing of the difference between the two groups using resampling analysis. Post-logging samples in the temporal reference lakes were deleted from the analysis. **, $p < 0.01$.

Fig. 3. Percent organic matter (open circles, lower scale) and cutting history (black bars, upper scale) for reference (*a–d*) and impact (logged, *e–h*) lakes: reference lakes: (*a*) Angora, (*b*) Blue, (*c*) Little Toquart, and (*d*) Toquart; logged lakes: (*e*) Black, (*f*) Maggie, (*g*) Pachena, and (*h*) Sugsaw. Total percentage of watershed cut is given in Table 1.



with the onset of logging, whereas many of the littoral genera (e.g., *Eunotia*, *Achnanthes*, *Cymbella*, and *Brachysira*) decrease. In Maggie and Sugsaw lakes (Fig. 5*b* and 5*d*), the significant changes in diatom species composition after logging are the result of minor differences in the mean abundance of many taxa. In Maggie Lake, there were increases in littoral

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Fig. 4. Diatom taxa with frequencies greater than 2% in at least two samples in the reference lakes, Angora (*a*), Blue (*b*), Little Toquart (*c*), and Toquart (*d*). Taxa are ordered according to their contribution to the average dissimilarity (SIMPER score) between the two groups (before and after 1950, excluding samples above the dotted line, which denotes the onset of logging). Consequently, taxa showing the largest changes before and after 1950 are arranged from left to right. Note the different percentage scales for the various taxa.

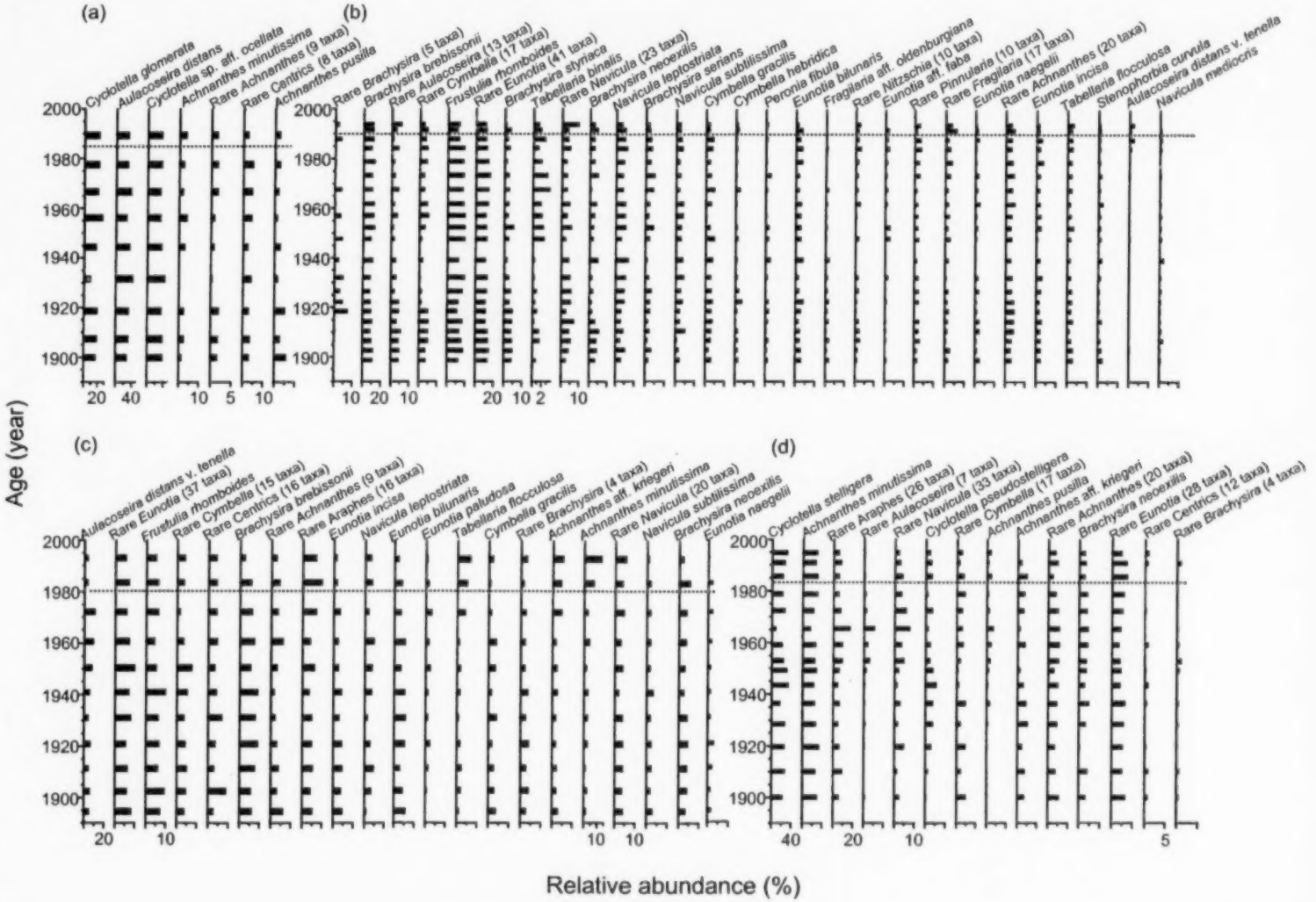

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Fig. 5. Diatom taxa with frequencies greater than 2% in at least two samples in the impact lakes, Black (*a*), Maggie (*b*), Pachena (*c*), and Sugsaw (*d*). Taxa are ordered according to their contribution to the average dissimilarity (SIMPER score) between the two groups (before and after the onset of logging, denoted by the dotted line). Consequently, taxa showing the largest changes before and after logging are arranged from left to right. Note the different percentage scales for the various taxa.

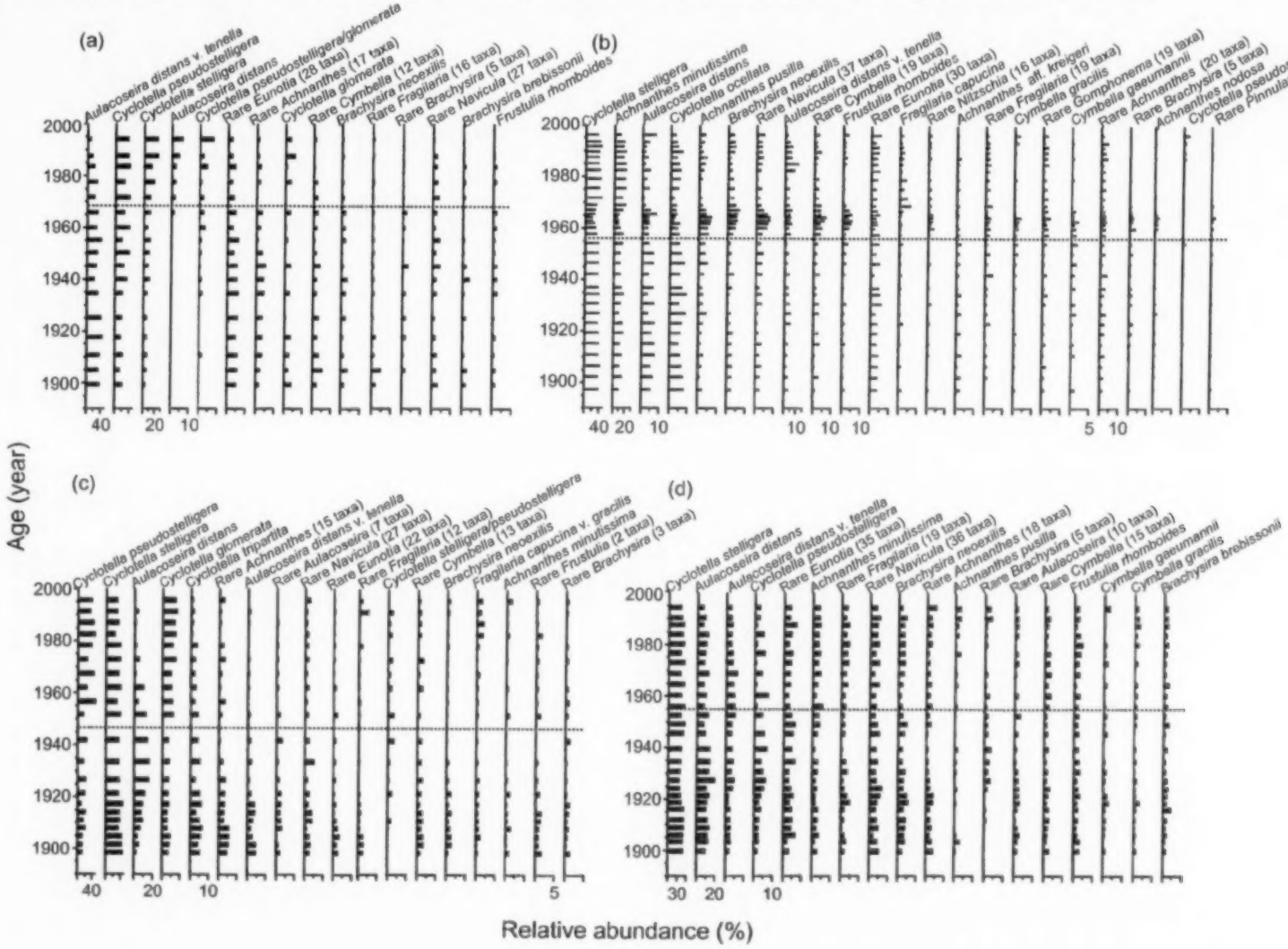

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Table 3. Results of ANOSIM (analysis of similarity).

	Reference lakes				Impact lakes			
	Angora	Blue	Little Toquart	Toquart	Black	Maggie	Pachena	Sugsaw
n (before/after)	5/3	11/10	6/3	7/5	12/5	15/18	11/10	16/11
R	0.2	0.121	0.66	0.186	0.669	0.293	0.68	0.129
p	0.179	0.078	0.012*	0.086	0.001**	0.001**	0.001**	0.001**

Note: Post-logging samples in the temporal reference lakes were deleted from the analysis. The number of samples (*n*) is given for each group: before and after the onset of logging in the impact lakes and before and after 1950 in the reference lakes (the date of the onset of logging in 3 of the 4 impact lakes). *, $p < 0.05$; **, $p < 0.01$.

taxa, such as *Achnanthes pusilla* Grunow in Cleve and Grunow, *Brachysira neoexilis* Lange-Bertalot, *F. rhomboides*, *Navicula* spp., and *Cymbella* spp. (Fig. 5*b*), associated with the peak in organic matter in the early 1960s (Fig. 3*f*). This may be a reflection of the transport of more littoral sediment, associated with an erosional event, reaching the center of the lake (see Discussion).

In the one reference lake (Little Toquart) that showed significant changes in diatom species composition, there was an increase in the abundance of *A. distans* var. *tenella* and a decrease in *F. rhomboides* after 1950 (excluding the two samples after the onset of logging in ~1980), with changes in other taxa being quite subtle (Fig. 4*c*). Although not included in the analyses presented here, after the onset of logging in Little Toquart Lake, there was a distinct increase in *Tabellaria flocculosa* (Roth) Kützing and *A. minutissima*. The other three reference lakes did not show a significant difference in species assemblages before and after 1950. However, in Toquart Lake (Fig. 4*d*), there was a distinct increase in *Aulacoseira* spp. from approximately 1950 to 1970 that corresponds to the large but unexplained peak in organic matter content (Fig. 3*d*).

Ordination results of the physical characteristics of the lakes

Exploratory PCA analysis of physical characteristics of the lakes did not produce any evidence for differences between the impact and reference lakes, based on their physical characteristics, that could explain the significant diatom changes seen primarily in the impact lakes. The exception was Angora Lake (reference lake), which has much higher slopes within its catchment than any other lake (Table 1) and thus separated out based on this characteristic; however, it did not show any significant changes in diatom species composition. PCA analysis of lake-water chemical variables was also explored, but no unique differences between the reference and impact lakes were found. However, since the chemical data were collected only after clear-cutting began in all lakes, and because the time since the last cut is grossly different between lakes, it is impossible to evaluate the importance or cause of any chemical differences between the lakes with respect to forestry activities.

CVA analysis of two groups of lakes (those with a significant response of diatoms and a high *R* statistic (two impact lakes: Black and Pachena; one reference lake: Little Toquart) and those with a low *R* statistic) could not explain the different diatom responses based on any of the physical characteristics of the lakes and their associated watersheds. This may, in part, be due to the small number of lakes examined, the limited number of physical variables included in the analysis, and important variables, such as volume of the lake (unknown for two of the eight lakes) and residence time (unknown for all lakes), not being included.

Discussion

The before–after design of this study in both impact and reference lakes from the same biogeoclimatic setting is a means of evaluating changes due to forestry activities, while at the same time attempting to assess if changes may be occurring at the local–regional scale in the absence of forest-harvesting activities. A similar study in Ontario by Paterson et al. (1998) illustrated the importance of this type of design. These authors observed changes in chrysophyte assemblages in both impact and reference lakes, and concluded that the observed changes may be the result of a regional drought in the area and thus may not be due solely to forestry activities or wildfire. In our study, the degree of change in the diatom assemblages was, on average, greater in the impact lakes than in the reference lakes, with the exception of one reference lake. This suggests that forest harvesting has had an impact on lakes that is larger than that seen in most reference lakes, but that natural variability can be as large as the changes seen before and after the onset of logging.

The magnitude and frequency of natural disturbances such as fire and windthrow in a forested region are driven principally by climate and physiography. For example, on the drier east coast of Vancouver Island, fires occur more frequently than on the west coast, where climate is wetter and windthrow and debris slides are the dominant natural disturbances (Clayoquot Sound Scientific Panel 1995). Short-term chemical responses to both windthrow and fire have been recorded in the streams of the Experimental Lakes Area in Ontario (Schindler et al. 1980; Bayley et al. 1992). Schindler et al. (1980) found that the effects on stream runoff and nutrient yields from windthrow disturbance fell within the same range as those from fire, with conditions returning to pre-disturbance levels within 3 years. Although Bayley et al. (1992) found elevated levels of nitrogen (N) and phosphorus (P) in the streams for several years following two fires 6 years apart, the total N and P inputs into Lake 239 were only slightly elevated.

Longer-term studies of natural disturbance on lake ecosystems are rare, but paleolimnological techniques have been used to detect natural forest disturbance from fires and windthrow. For example, Rhodes and Davis (1995) investigated the impact of watershed disturbance on a small oligotrophic pond in Maine over the last 3500 years by examining changes in geochemistry and organic matter (decreases indicating brief pulses of soil erosion), as well as changes in pH

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inferred from changes in the species composition of diatoms. Increases in pH following disturbance were thought to be indicative of increased inputs of base cations from wood ash and mineral soils (Rhodes and Davis 1995). Similarly, the analysis of diatoms in the lake sediments of a small oligotrophic lake in Finland over the past 100 years showed a diatom-inferred increase in pH associated with a fire in 1890; however, the influence of climate on the observed changes could not be conclusively dismissed (Korhola et al. 1996). The apparent sensitivity of these lakes to pH changes associated with fire disturbance may, in part, be due to their poor buffering capacities and topographic location (both are headwater lakes), as opposed to differences in the ratio of watershed area to lake surface area (WA/LA) that differ by an order of magnitude (~50:1 for the Maine pond and ~3:1 for the Finland lake).

The WA/LA and catchment slope were proposed as characteristics that determined the magnitude of lake eutrophication in response to the hemlock decline (Hall and Smol 1993). Hall and Smol (1993) suggest that watersheds with steeper slopes may supply a greater proportion of mineral material to a lake basin than those with gentler slopes. The one impact lake (Pachena) that showed a significant decrease in organic matter after the onset of logging also had the steepest terrain among the impact lakes. The steepness of the terrain, along with a high density of roads (Spicer 1999), likely contributed to the higher proportion of mineral erosion in this basin. However, WA/LA and catchment slope do not appear to explain the significant changes seen in diatom assemblages, even in those lakes with the greatest changes (i.e., impact lakes Black and Pachena and reference lake Little Toquart). Little Toquart Lake is one of the shallowest of the study lakes, which may make it more sensitive to changes. However, Blue Lake (reference lake) is even shallower and did not show any significant change in diatom species composition. The organic-matter profile of Little Toquart Lake does not indicate any natural disturbance from windthrow or debris slides, and fire has not occurred in the basin over this time period. In the absence of such changes, the variability in diatom species may be related to in-lake processes (e.g., population dynamics), although the exact cause remains elusive.

Carignan et al. (2000) proposed an intuitively simple model to explain the degree of impact on water quality from forest harvesting on lakes that is based on the area of watershed cut and lake area. According to this model, the greater the ratio of area harvested divided by either the lake's volume or its surface area, the greater the impact. Applying this model to our study lakes, the greatest degree of impact should have been seen in Maggie and Sugsaw lakes, whereas, in fact, these are the two lakes with the lowest R statistics and therefore the least impacted. The Carignan et al. (2000) model may not be applicable to our study region, for several reasons: (*i*) different physiographic and edaphic conditions; (*ii*) different time frames, since their model is based on 1 year of cut compared with several decades of cutting in this study; or (*iii*) uncertainties in their model related to deriving preharvest conditions on the water chemistry of the logged lakes from a set of reference lakes.

Although all four impact lakes showed a statistically significant change in diatom assemblage before and after the onset of logging, these changes were relatively subtle even in those lakes with the greatest changes (i.e., a high R statistic). The difference in diatom assemblages before and after forest harvesting typically resulted from small shifts in the percentages of taxa rather than from the appearance or disappearance of taxa. The small stratigraphic changes in diatom taxa reported here are in contrast with the large changes in species assemblages observed in studies of lake acidification (e.g., Cumming et al. 1994), eutrophication (e.g., reviewed in Hall and Smol 1999), and climate change (e.g., Laird et al. 1996). The lack of changes of greater magnitude could be due to several reasons, including the short-term changes, typically in the order of a few years, seen in water chemistry from monitoring studies following forest harvesting, (e.g., Likens et al. 1978; Feller and Kimmins 1984; Hornbeck et al. 1986), and a limited export of nutrients, particularly P.

Podzols, the dominant soil type on the west coast of Vancouver Island, binds P tightly, owing to the low soil pH, and thus release to streams is minimal (Clayoquot Sound Scientific Panel 1995). In general, P is less susceptible to leaching than N (Keenan and Kimmins 1993). Even after whole-tree harvest in a small watershed at the Hubbard Brook Experimental Forest in New Hampshire, negligible P was exported to streams while other nutrients increased dramatically (Yanai 1998). It is well known that P is the primary limiting nutrient in freshwater lakes, and increased loading of P from forest harvesting could result in eutrophication (Likens et al. 1978), with eutrophication resulting in changes to the diatom assemblage (e.g., see review by Hall and Smol 1999). However, clear-cut logging and burning in Carnation Creek, located on the west coast of Vancouver Island near our Bamfield study sites, had only a small impact on periphyton (composed primarily of diatoms) production (Hartman and Scrivener 1990). Short-term responses were seen at some sites during the second year of post logging that were potentially associated with increased P. In areas with richer soils, land clearance associated with logging and later settlement can result in eutrophication and distinct diatom changes, as seen in the study by Fritz et al. (1993).

Although P is typically the limiting nutrient in freshwater lakes, other chemical factors can greatly affect diatoms and other algae in the lakes. Diatom-inferred pH increases have been linked to fire (e.g., Rhodes and Davis 1995) and land clearance associated with agriculture (Renberg et al. 1993), as well as to logging, in some studies (e.g., Davis et al. 1994). All the lakes in these studies were acidic lakes (pH < 6.0), typically with shallow soils in the watershed and, thus, probably with low buffering capacities. The lakes in our study region are generally circumneutral (pH = 6.5–7.8) and, thus, have greater buffering capacities than those in the studies above, which may, in part, account for the minor diatom changes seen in this region. At this time, we cannot infer whether the diatom changes seen in the logged lakes are due to changes in chemical variables such as pH, P, or conductivity. Although an extensive calibration set of chemical variables and diatom taxa exists for over 200 lakes in the interior of British Columbia (Wilson et al. 1996), many of the diatom taxa seen in the Vancouver Island lakes are either not present or are of low occurrence in this dataset, owing to the present paucity of low conductivity oligotrophic lakes in the

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calibration set. Regardless, the relatively minor changes in diatom taxa suggest that diatom-inferred changes in water chemistry associated with logging would be minimal.

Another reason for the small-magnitude changes seen in the diatom assemblages may be related to the rate of revegetation. The rate of recovery of chemical and streamflow changes is strongly linked to the rate of revegetation in the watershed (Marks and Bormann 1972; Likens et al. 1978). Given the high precipitation regime in our study region, and assuming minimal soil disturbance, it is likely that the rate of revegetation would be quite rapid, which would help to account for the lack of response in many lakes. In addition, in any particular year, the percentage of the watershed cut was usually less than 10% and not more than 20%, which may have reduced the impact.

In summary, the minor changes, albeit statistically significant, seen in the diatom assemblages after substantial logging in the watersheds may be due to several reasons, including: (*i*) our average sampling resolution of 5–10 years may have been too long to detect short-term changes in water chemistry; (*ii*) the soils in the watersheds of our lakes generally contained low levels of P; (*iii*) our study lakes were generally well buffered and thus not susceptible to large changes in pH associated with land disturbance; and (*iv*) rates of revegetation were high and only a low percentage of watershed was cut in any particular year. The significant changes in diatom species composition seen in one reference lake suggest that natural variability can be as great as the variability seen in the impact lakes, and implies that some of the changes seen in the impact lakes may, in part, be due to natural variation. However, the other three reference lakes did not show any significant changes, suggesting that a regional signal is not complicating the impacts from forest harvesting, but rather that there is a local signal affecting the one reference lake.

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A regional paleolimnological assessment of the impact of clear-cutting on lakes from the central interior of British Columbia

Kathleen Laird and Brian Cumming

Abstract: The impact of forest harvesting on lakes within the central interior of British Columbia was examined in a paleolimnological study of six lakes that had 28–82% of their watersheds clear-cut (impact lakes) and four lakes that had experienced no logging in their watersheds (reference lakes). Changes in diatom species composition and percent organic matter in ^{210}Pb-dated sediment cores were compared over the last 80 years in each of the impact lakes before and after the onset of forest harvesting and, in the reference lakes, before and after 1960 (the average onset of logging in five of the six impact lakes) and before and after 1975 (the onset of logging in one impact lake). Significant changes in species composition of diatoms following forest-harvesting activities were detected in four of the impact lakes and three of the reference lakes; however, the changes in diatom species composition were small, with changes in the relative abundance of the most common species being at most 11%. Significant increases in the percent organic matter after 1960 were found in one impact lake and three reference lakes; again these changes were small, with increases of 2–5%.

Résumé : L'étude paléolimnologique de six lacs dont 28–82% du bassin versant a été soumis à la coupe à blanc (lacs affectés) et de quatre autres lacs dont le bassin versant n'a pas été modifié (lacs témoins) a permis d'évaluer l'impact de la coupe de la forêt sur les lacs dans la région intérieure centrale de la Colombie-Britannique. Les changements dans la composition spécifique des diatomées et dans le pourcentage de matière organique dans des carottes de sédiments datés au ^{210}Pb ont été comparés au cours des 80 dernières années, soit dans chacun des lacs affectés avant et après la coupe et dans les lacs témoins, avant et après 1960 (la date moyenne du début de la coupe dans cinq des six lacs affectés), de même qu'avant et après 1975 (la date du début de la coupe dans un des lacs affectés). Des changements significatifs dans la composition spécifique des diatomées après la coupe ont été trouvés dans quatre des lacs affectés et trois des lacs témoins; cependant, ces modifications étaient faibles, les variations dans la composition relative des espèces communes atteignant tout au plus 11%. Des augmentations significatives du pourcentage de matière organique après 1960 ont été observées dans un lac affecté et trois lacs témoins; ces hausses étaient faibles, de l'ordre de 2–5%.

[Traduit par la Rédaction]

Introduction

The impact of forest-harvesting activities on aquatic environments has been well documented in many streams. Forest clearing may result in increased streamflow primarily during peak spring flows and the growing season as a result of a reduction in transpiration and canopy interception (Bormann and Likens 1979; Hornbeck et al. 1986). The magnitude of change in streamflow depends on the annual precipitation of the region, as well as on precipitation during the years following the cutting (Cheng 1989; Stednick 1996), with recovery to prelogging conditions being dependent on the rate of revegetation. Forest clearing may also affect the turbidity of streams, depending on the degree of soil disturbance (Keenan and Kimmins 1993). Generally, concentrations of dissolved ions in streams increase for several years following forest harvesting (e.g., Feller and Kimmins 1984; Hornbeck et al. 1986; Jewett et al. 1995), as does temperature, with the degree of change being dependent on whether a buffer strip is retained along the streams and on the percentage of the watershed harvested (Hartman and Scrivener 1990; Keenan and Kimmins 1993).

The impacts of forest harvesting on lakes is grossly understudied in comparison with the research on streams. Rask et al. (1998) found minor responses in water quality, plankton, zoobenthos, and fish to forest harvesting in three lakes in Finland, in comparison with one reference lake, over 4 years, including 1 year of preharvest data. Patoine et al. (2000) found short-lived impacts (<3 years) of fire and clear-cutting on zooplankton biomass in a suite of lakes in southern Quebec. These studies, as well as most stream studies, are of relatively short duration (<5–10 years) and typically lack data on the natural variability of the aquatic system prior to the forest-harvesting activities. In a recent special issue on watershed perturbations (Carignan and

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Steedman 2000), only two of the seven studies that examined the impacts of forest harvesting on limnological variables of lakes had any preharvest data.

Analysis of sediment records from lakes provides a reliable archive of past environmental and ecological changes and can be used as a means of assessing the impacts associated with forestry activities over a longer time frame. For example, in northwestern Ontario lake sediments were used to assess the impacts of logging, fire, and climate on impact and reference lakes over the past 30 years (Blais et al. 1998; Paterson et al. 1998). In Finland, lake-sediment records were used to examine the impacts of logging and peatland management over the past 100–150 years (Simola et al. 1995; Turkia et al. 1998). Studies in the United States assessed the impact of logging and human settlement on lake ecosystems using paleolimnological techniques (e.g., Fritz et al. 1993; Lott et al. 1994).

The purpose of this study was to examine the impacts of forest-harvesting activities on lakes in the central interior region of British Columbia. Changes in the sediment organic matter and diatom assemblages preserved in the lake sediments over the last 80 years were compared within six lakes before and after the onset of forest harvesting (impact lakes) and in four lakes in the same region that had no logging (reference lakes). Diatom analysis was the main focus of this study, because they are one of the most widely used and sensitive indicators of historic changes in lake water conditions (e.g., Stoermer and Smol 1999). This study was designed to detect possible changes associated with logging practices that occurred over years to decades, and allows an assessment of the extent of natural variability in the reference lakes and in the impact lakes prior to clear-cutting. Furthermore, changes in the impact lakes (post logging), in comparison with the reference lakes, can be assessed to determine if the changes in the impact lakes are likely attributable to clear-cutting or are related to more regional changes, such as climate. A similar study was carried out on the west coast of Vancouver Island and is presented elsewhere (Laird et al. 2001, this issue).

Materials and methods

Study area, sites, and study design

The lakes examined in this study are located on the central interior plateau of British Columbia near the town of Prince George (Fig. 1). This region lies within the Sub-boreal Spruce biogeoclimatic zone and is dominated by white spruce (*Picea glauca* (Moench) Voss) and lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm), as well as by stands of trembling aspen (*Populus tremuloides* Michx.). Sub-alpine fir (*Abies lasiocarpa* (Hook.) Nutt.) is common in the understory (Valentine et al. 1978). Average rainfall in Prince George is 620 mm, occurring primarily between May and October, while average snowfall is approximately 230 cm, occurring from November to March (Valentine et al. 1978). Dominant soils are gray luvisols, composed of a thin poorly decomposed layer of organic matter and an upper mineral layer of grayish brown silt and fine sand (Valentine et al. 1978). Slopes in the catchments of the lakes range from gentle to moderate steepness (Table 1).

The lakes in this study were selected from government databases (e.g., Ministry of Forests files, Ministry of Environment fisheries inventory files, maps, and air photos), based on the following criteria: (*i*) the presence of both reference and logged lakes within an area of similar precipitation, geology, and vegetation; (*ii*) the absence of known large-scale natural disturbances, such as fire, in the last 100 years; and (*iii*) similarity in the range of physical (e.g., lake area, watershed area, depth, simple bathymetry) and chemical (e.g., pH, conductivity, nutrients) characteristics between impact and reference lakes (Table 1). Ten lakes were analyzed in three categories: (1) four reference lakes (no logging within the watershed); (2) three low-cut lakes (total cut in the watershed approximately 30%); and (3) three high-cut lakes (total cut in the watershed approximately 45–82%). The original surveys of the reference watersheds did not indicate the occurrence of extensive fires. However, after site selection, detailed analysis of forest cover and stand ages indicated stand ages of less than 100 years in the reference watersheds (Spicer 1999), suggesting potential fire or other disturbance, such as insect infestation or windthrow, since the early 1900s. The percent of the watershed with stand ages of less than 100 years was ~80% in Justine Lake, ~40% in Unnamed and Secord lakes, and ~6% in Boomerang Lake.

Field sampling

Lakes without road access were sampled from a Beaver float plane out of Prince George, British Columbia, whereas the other lakes were sampled by canoe. Two cores were retrieved from the deep basin of each lake, using a modified K-B gravity corer (Glew 1989) equipped with a 60 cm long core tube (internal diameter ~7.6 cm). The cores ranged in length from 30 to 50 cm, with an average length of approximately 40 cm, and were secured upright in buckets with padded bottoms during transport by plane or were sectioned vertically directly on the lake shore. All cores were sectioned into polyethylene whirlpack bags within 24–36 h of retrieval. Each core was sectioned every 0.25 cm for the top 40 cm, and every 0.5 cm thereafter. Core samples from each lake were placed in large plastic bags and stored in a coldroom at the University of Northern British Columbia prior to being shipped on ice to Queen's University, where they were stored at 4°C.

Sample collection and preparation

Collection and analysis of water samples, preparation and analysis of ^{210}Pb and organic-matter samples, and diatom preparation and enumeration followed Laird et al. (2001, this issue), with the exception of the few details given below that are specific for this study region. From 15 to 18 samples from one core from each lake were analyzed for ^{210}Pb. The second core from two randomly chosen lakes in each of the three groups (reference, low cut, and high cut) was analyzed for ^{210}Pb activity, to determine the reproducibility of the ^{210}Pb profiles. Percent organic matter was determined every 0.5-cm interval, an approximately 1- to 3-year resolution, from 1920 to present.

Statistical analyses

Statistical analyses follow Laird et al. (2001, this issue), with the following exceptions that are specific for this study region. The samples for diatom and percent organic matter analysis from each of the logged lakes were split a priori into two groups according to ^{210}Pb estimated dates: those intervals that occurred before the onset of logging and those that occurred after logging. The approximate date of the onset of logging for five of the six impact lakes is 1960. The onset of logging in the sixth lake is 1975. The diatom samples for each of the reference lakes were split a priori into those before and after 1960 and those before and after 1975. Samples analyzed for percent organic matter for each of the reference lakes were split a priori into those before and those after 1960.

Principal components analysis (PCA) was carried out on the same physical characteristics of lakes and watersheds (maximum depth, lake area, watershed area, watershed/lake area, total stream

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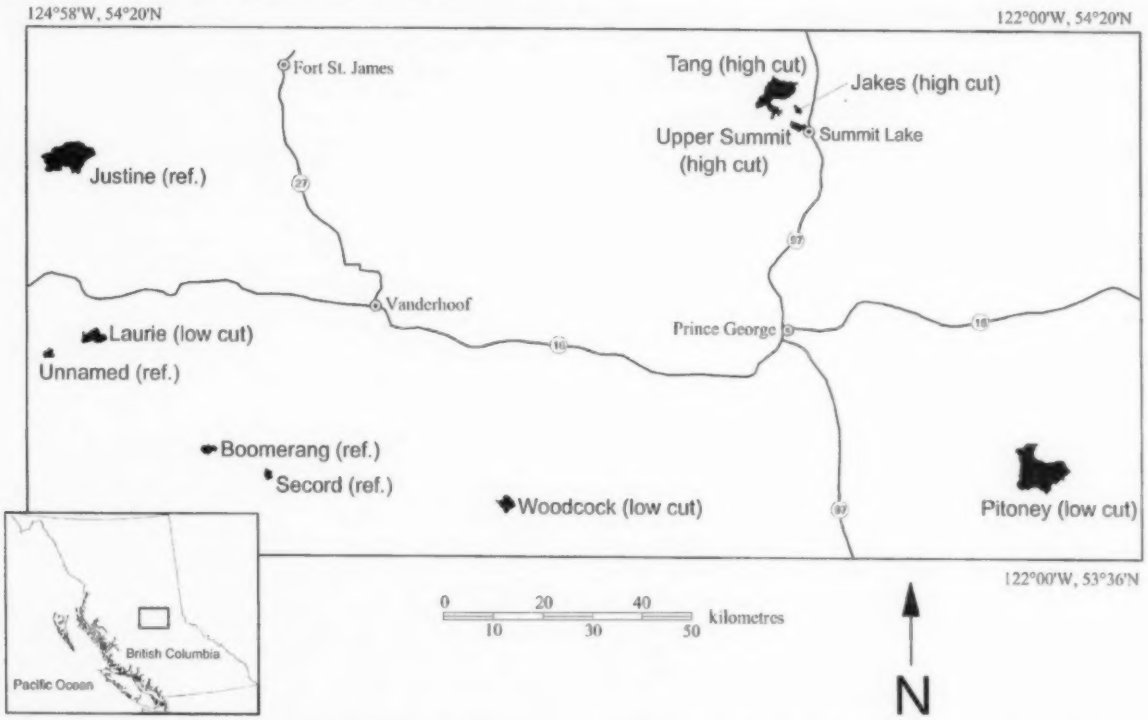
Fig. 1. Location of the watersheds for the reference (ref.) and impact (low cut, high cut) lakes. Approximate latitude and longitude are given in the corners of the diagram. Modified from Spicer (1999).



length, and slope) as in the Vancouver Island dataset (Laird et al. 2001, this issue), and on additional physical characteristics (elevation, mean depth, and volume) that were available for the central interior lakes. All variables, except elevation, depth, and slope, were log-transformed to meet the assumptions of normality.

Results

^{210}Pb profiles

The ^{210}Pb profiles from the reference and impact lakes all show decreases in activity with depth and all indicate that the cores were of sufficient length to reach supported levels of ^{210}Pb activity (Fig. 2). Log of total ^{210}Pb versus cumulative dry weight was plotted, to indicate the values from which supported ^{210}Pb were calculated (Fig. 2). Plots of log unsupported ^{210}Pb show similar trends to those presented and, for most cores, are indistinguishable post 1920 A.D. from log of total ^{210}Pb, owing to the small and constant amount of supported ^{210}Pb. Maximum activities in the majority of the cores ranged between 0.3 and 1.0 Bq·g^{-1}, with the exception of Jakes Lake, which had much higher surficial activities (1.8 Bq·g^{-1}). Several cores (e.g., Figs. 2*a*, 2*b*, 2*f*, and 2*g*) had nearly constant activities at the top of the cores, indicating either increased sedimentation rates or mixing in the uppermost sediments. The time frame of interest in this study (post 1920) is well represented in the unsupported region of the ^{210}Pb profiles, a time period that generally experienced relatively small changes in the sedimentation rates or potential mixing of sediments in both the reference and impact lakes (Fig. 2), with the exception of the cores mentioned above. Even though some ^{210}Pb profiles suggest that either changes in sedimentation rate or mixing occurred, this does not hamper our conclusions, because this study was designed to detect changes associated with logging practices occurring over years to decades, and our conclusions are drawn from all lakes, which show similar trends in species changes (see below).

Reproducibility of the ^{210}Pb profiles in replicate cores was generally quite good (Figs. 2*a*, 2*d*–2*f*, 2*i*, and 2*j*), but not as precise as seen in the cores from Vancouver Island (Laird et al. 2001, this issue). A number of the cores collected from lakes in the central interior (e.g., Jakes (Fig. 2*h*), Upper Summit (Fig. 2*j*), and Woodcock (Fig. 2*g*)) had extremely high water content throughout the entire core (94–98% water). Consequently, sectioning the cores from these lakes was difficult in comparison with sectioning those from the other interior lakes and from lakes on Vancouver Island, which had much lower water content (e.g., reaching 70–88% water downcore; Laird et al. 2001, this issue). In Pitoney Lake (Fig. 2*f*), the discrepancy between the two profiles is most pronounced in the uppermost sample, a phenomenon that is not uncommon in many lakes. Overall, the ^{210}Pb results obtained from the reference and impact lakes suggest that reliable chronologies can be established from these cores.

Organic matter and diatom species changes

Resampling techniques were used to test the hypothesis that there was no difference in the mean abundance of organic matter before and after the onset of clear-cutting, or before and after 1960 in the reference lakes. An average of

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Table 1. Summary of the physical and chemical variables of the study lakes.

	Reference lakes				Impact lakes					
					Low cut			High cut		
	Boomerang	Justine	Secord	Unnamed	Laurie	Pitoney	Woodcock	Jakes	Tang	Upper Summit
Lake elevation (m asl)	1140	820	1220	1100	940	900	920	760	720	720
Lake area (km^2)	0.52	2.48	0.4	0.79	1.0	1.76	1.33	0.58	0.44	0.97
Watershed area (km^2)	3.9	43.7	2.2	2.1	9.3	60.3	5.6	1.2	30.9	3.3
Watershed cut (km^2)	0.0	0.0	0.0	0.0	2.8	20.8	1.56	0.82	14.0	2.7
Watershed cut (%)	0.0	0.0	0.0	0.0	30.2	34.5	27.7	68.2	45.3	81.7
Watershed/lake area	7.5	17.6	5.5	2.7	9.3	34.3	4.2	2.1	70.2	3.4
Mean depth (m)	2.5	5.7	2.5	6.9	9.9	8.5	6.8	6.5	5.7	3.5
Maximum depth (m)	11	10	8	19.5	23	19.8	15	15	10.9	6.4
Volume (10^6 m^3)	1.27	13.2	1.04	4.52	10.5	15.8	8.96	2.66	2.57	3.33
Mean watershed slope	0.034	0.047	0.042	0.093	0.072	0.090	0.038	0.053	0.026	0.011
pH	7.1	7.1	7.0	7.5	7.6	6.7	7.8	7.6	7.4	7.6
Specific conductivity ($\mu S \cdot cm^{-1}$)	50	59	55	68	65	30	118	57	47	78
Alkalinity ($mg \cdot L^{-1}$)	25.3	26.8	21.5	34.0	27.6	15.4	63.9	29.4	23.8	40.3
Total phosphorus ($\mu g \cdot L^{-1}$)	15	27	15	18	11	15	7	29*	11	6
Total nitrogen ($\mu g \cdot L^{-1}$)	410	470	350	320	330	290	360	—	—	400
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	7.4	12	7.0	5.7	7.4	3.7	7.1	6.7	8.0	3.6
Silica ($mg \cdot L^{-1}$)	5.0	7.2	5.1	3.2	1.1	5.5	0.6	0.6	3.6	0.4
Calcium ($mg \cdot L^{-1}$)	6.5	7.6	4.1	9.6	7.1	3.6	14.9	7.0	5.7	8.7
Sodium ($mg \cdot L^{-1}$)	2.7	1.8	1.4	1.7	2.3	0.4	2.8	0.9	0.7	1.5
Magnesium ($mg \cdot L^{-1}$)	1.4	1.5	2.0	1.4	1.1	1.3	5.5	2.3	2.0	4.4
Dissolved inorganic carbon ($mg \cdot L^{-1}$)	4.9	5.5	4.4	7.4	6.0	2.9	14.0	6.3	4.7	8.5
Dissolved organic carbon ($mg \cdot L^{-1}$)	15.3	17.0	12.3	6.9	9.4	10.0	10.2	6.7	12.4	12.1

Note: Chemical variables are based on one sample taken from a deepwater station in July 1997. Mean watershed slope is calculated by H/L, where H is the difference between the highest and lowest point in the basin and L is the horizontal distance along the longest dimension of the basin parallel to the principal drainage line (Schumm 1956). Anomalously low total nitrogen values for two lakes are believed to have been the result of analytical error and were deleted (indicated by "—"). An asterisk (*) indicates a total phosphorus (TP) value from a littoral station; the deepwater value is believed to be anomalously low, owing to analytical error and was deleted. There is a high correlation between the deepwater and littoral-station TP values from 41 lakes in this region ($r = 0.97$; B. Cumming unpublished data). Abbreviations: asl, above sea level.

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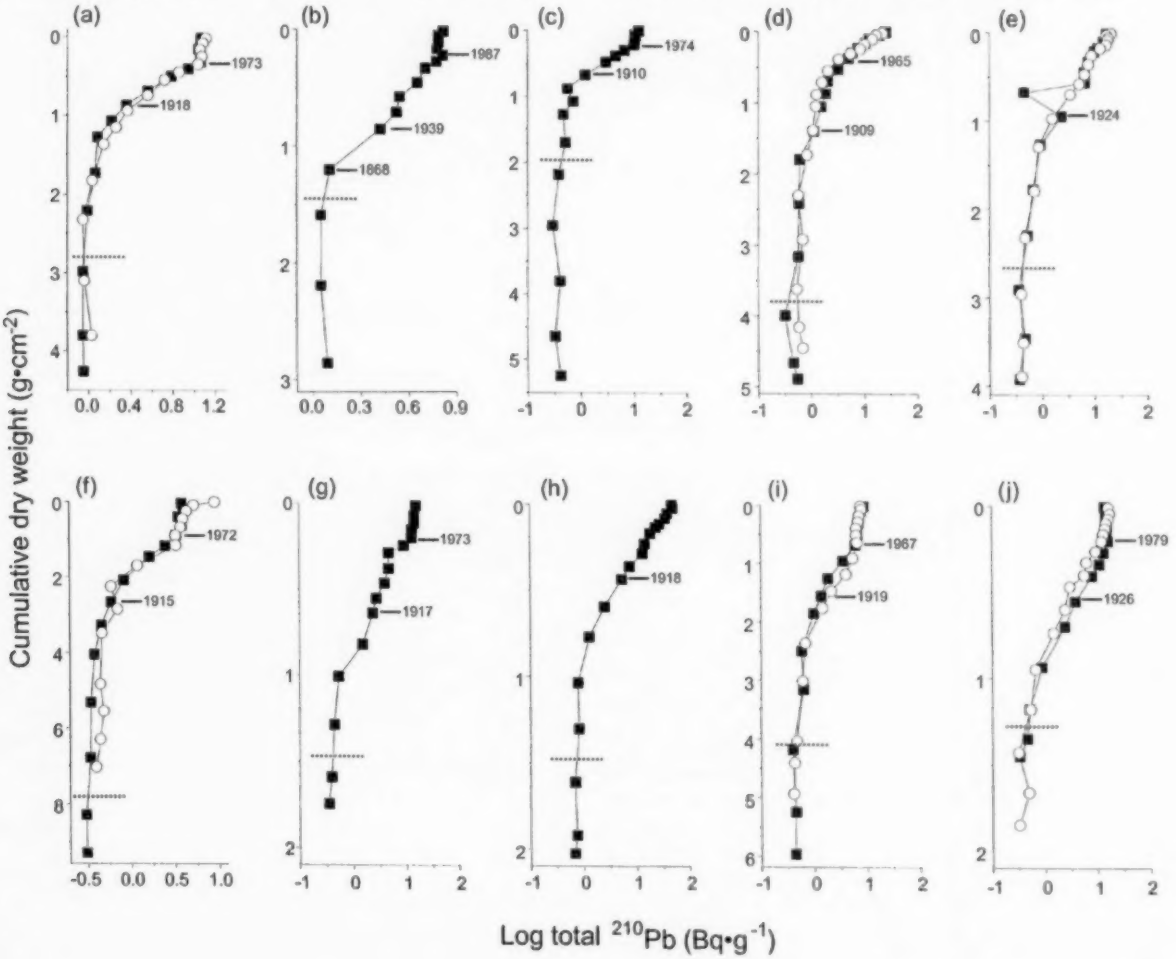
Fig. 2. Log of total ^{210}Pb activity versus cumulative dry weight for reference (*a–d*) and impact (logged, *e–j*) lakes: reference lakes: (*a*) Boomerang, (*b*) Justine, (*c*) Secord, and (*d*) Unnamed; low-cut lakes: (*e*) Laurie, (*f*) Pitoney, and (*g*) Woodcock; high-cut lakes: (*h*) Jakes, (*i*) Tang, and (*j*) Upper Summit. Core analyses were completed for profiles shown using solid squares. In six lakes, the second core retrieved from the lake was dated (open circles). Dates estimated from ^{210}Pb analysis are given for points of change in slope and the closest date to the beginning date of focus of this study (1920). Supported ^{210}Pb is indicated as samples below the dotted horizontal line.



40 samples per lake, giving a resolution between samples of 1–3 years, were analyzed for percent organic matter (Fig. 3). An approximately equal number of samples were analyzed before and after 1960, with the exception of Woodcock Lake, for which more samples were analyzed before harvesting, as logging did not begin there until 1975 (Table 2).

Analysis of similarity (ANOSIM) was used to test the hypothesis that there was no difference in diatom species assemblages before and after the onset of forest harvesting in each impact lake (1960 for five of the impact lakes and 1975 for the sixth) and before and after 1960 and before and after 1975 in each reference lake. An average of 14 samples per lake were analyzed for diatoms, giving an approximately equal number of samples before and after the onset of logging for the impact lakes or before and after 1960 for the reference lakes, and an average resolution between samples of 5–10 years (Figs. 4, 5, 6). The average number of diatom taxa in all lakes is 77, ranging from 41 in Unnamed Lake (Fig. 4*d*) to 118 in Tang Lake (Fig. 6*c*). Dominant taxa in many of the lakes include the littoral taxa *Fragilaria pinnata* Ehrenberg and *Fragilaria construens* (Ehrenberg) Grunow, which comprise approximately 30–70% of the diatom assemblages. Common planktonic taxa include *Cyclotella glomerata* Bachmann, *Cyclotella pseudostelligera* Hustedt, and *Asterionella formosa* Hassall, which reach abundances of 10–30% in many of the lakes. In Unnamed Lake, the meso-eutrophic planktonic taxa *Fragilaria crotonensis* Kitton and *Aulacoseira subarctica* (O. Müller) Haworth (Fig. 4*d*) are dominant. The number of taxa in the genera *Navicula* Bory and *Achnanthes* Bory was high in the study lakes, with the mean number of taxa in all lakes being 26 and 16, respectively; however, the percent abundance of individual taxa

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Fig. 3. Percent organic matter (open circles, lower scale) and cutting history, given as percent watershed cut per year (black bars, upper scale), for reference (*a–d*) and impact (logged, *e–j*) lakes: reference lakes: (*a*) Boomerang, (*b*) Justine, (*c*) Secord, and (*d*) Unnamed; low-cut lakes: (*e*) Laurie, (*f*) Pitoney, and (*g*) Woodcock; high-cut lakes: (*h*) Jakes, (*i*) Tang, and (*j*) Upper Summit. Total percentage of watershed cut is given in Table 1.

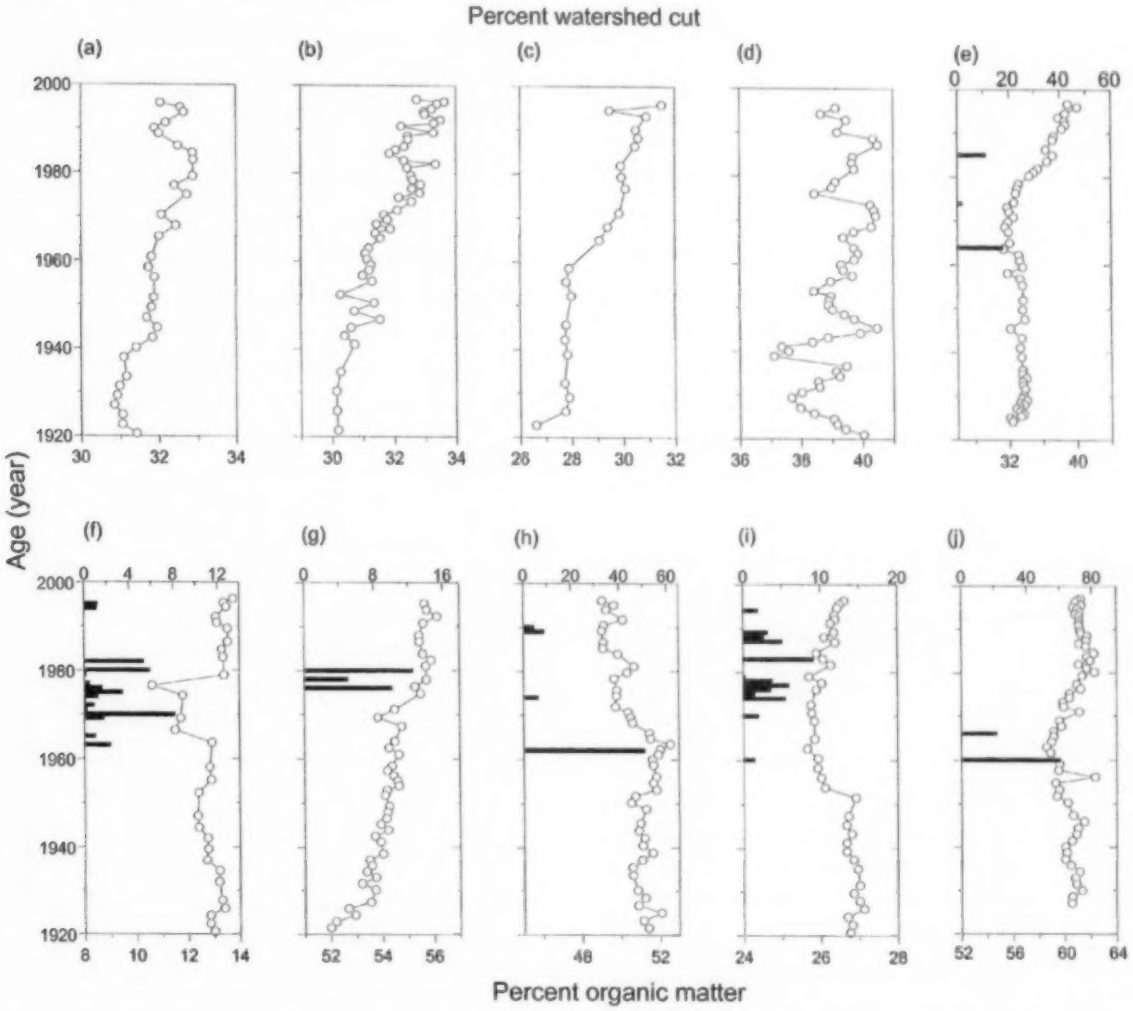

Table 2. Mean percent organic matter (OM) of samples before and after the onset of logging in the impact lakes and before and after 1960 in the reference lakes.

					Impact lakes					
	Reference lakes				Low cut			High cut		
	Boomerang	Justine	Secord	Unnamed	Laurie	Pitoney	Woodcock	Jakes	Tang	Upper Summit
Mean percent OM before	31.4±0.4	30.8±0.5	27.7±0.4	38.9±0.8	33.3±0.6	12.9±0.3	53.8±0.8	51.2±0.4	26.7±0.3	60.5±0.8
	(16)	(16)	(10)	(25)	(25)	(15)	(30)	(23)	(18)	(22)
Mean percent OM after	32.4±0.4	32.3±0.7	30.1±0.7	39.7±0.6	35.0±2.8	12.8±1.0	55.6±0.2	50.2±1.0	26.1±0.3	60.8±1.1
	(16)	(25)	(12)	(21)	(29)	(15)	(12)	(23)	(19)	(28)
t	6.95	7.66	10.00	3.64	2.86	0.08	8.17	–4.11	–6.13	0.73
p	0.026*	0.034*	0.001**	0.37	0.55	0.98	0.031*	0.29	0.08	0.88

Note: Values are mean ± 1 SD, with sample size (*n*) in parentheses. The *t* and *p* are given for testing of the difference between the two groups using resampling analysis. *, $p < 0.05$; **, $p < 0.01$.

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Fig. 4. Diatom taxa with frequencies greater than 2% in at least two samples for the reference lakes: (*a*) Boomerang, (*b*) Justine, (*c*) Secord, and (*d*) Unnamed. Taxa are ordered according to their contribution to the average dissimilarity (SIMPER score) between the two groups (before and after 1960). Consequently, taxa showing the largest changes before and after 1960 are arranged from left to right. Note the different percentage scales for the various taxa.

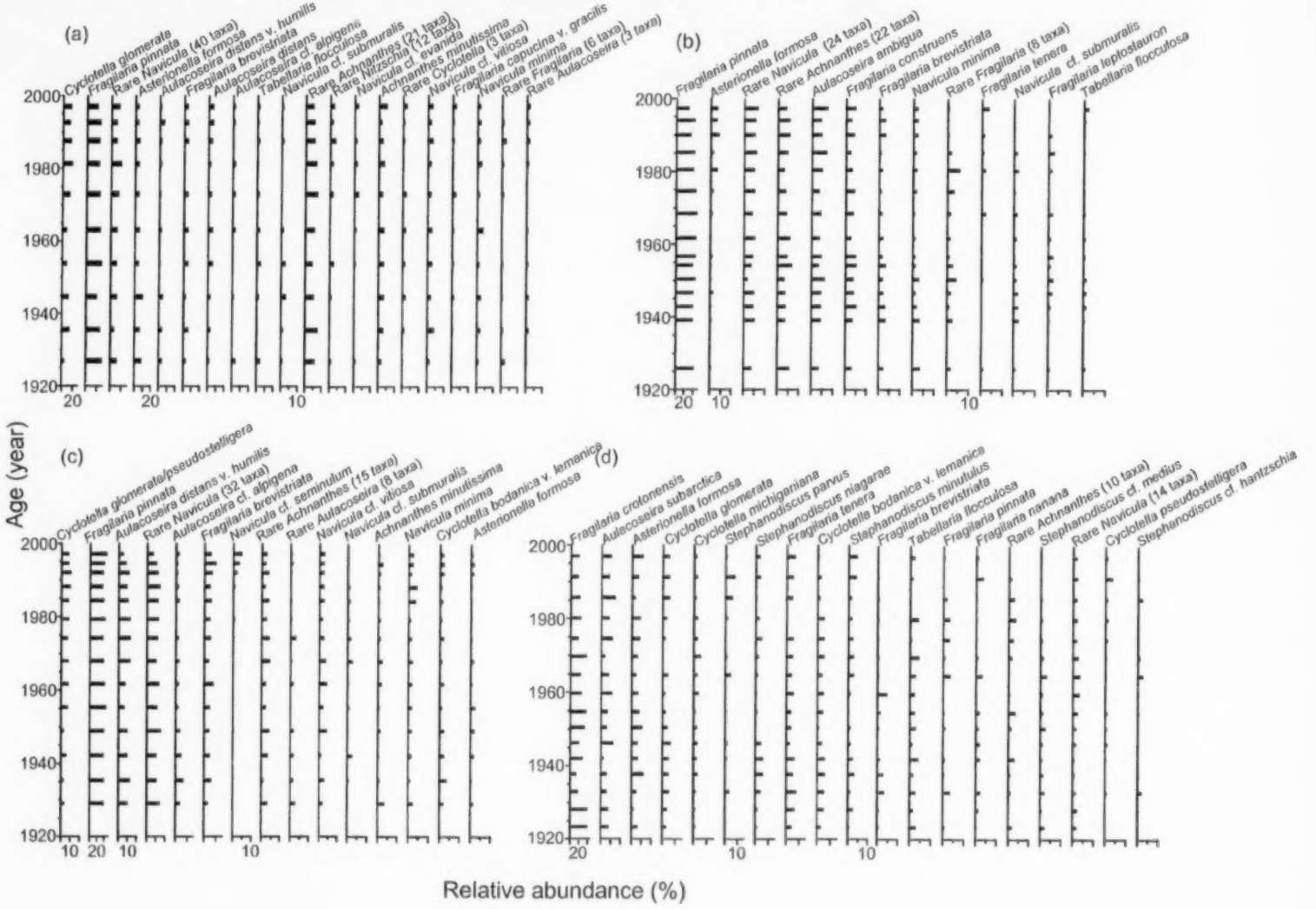

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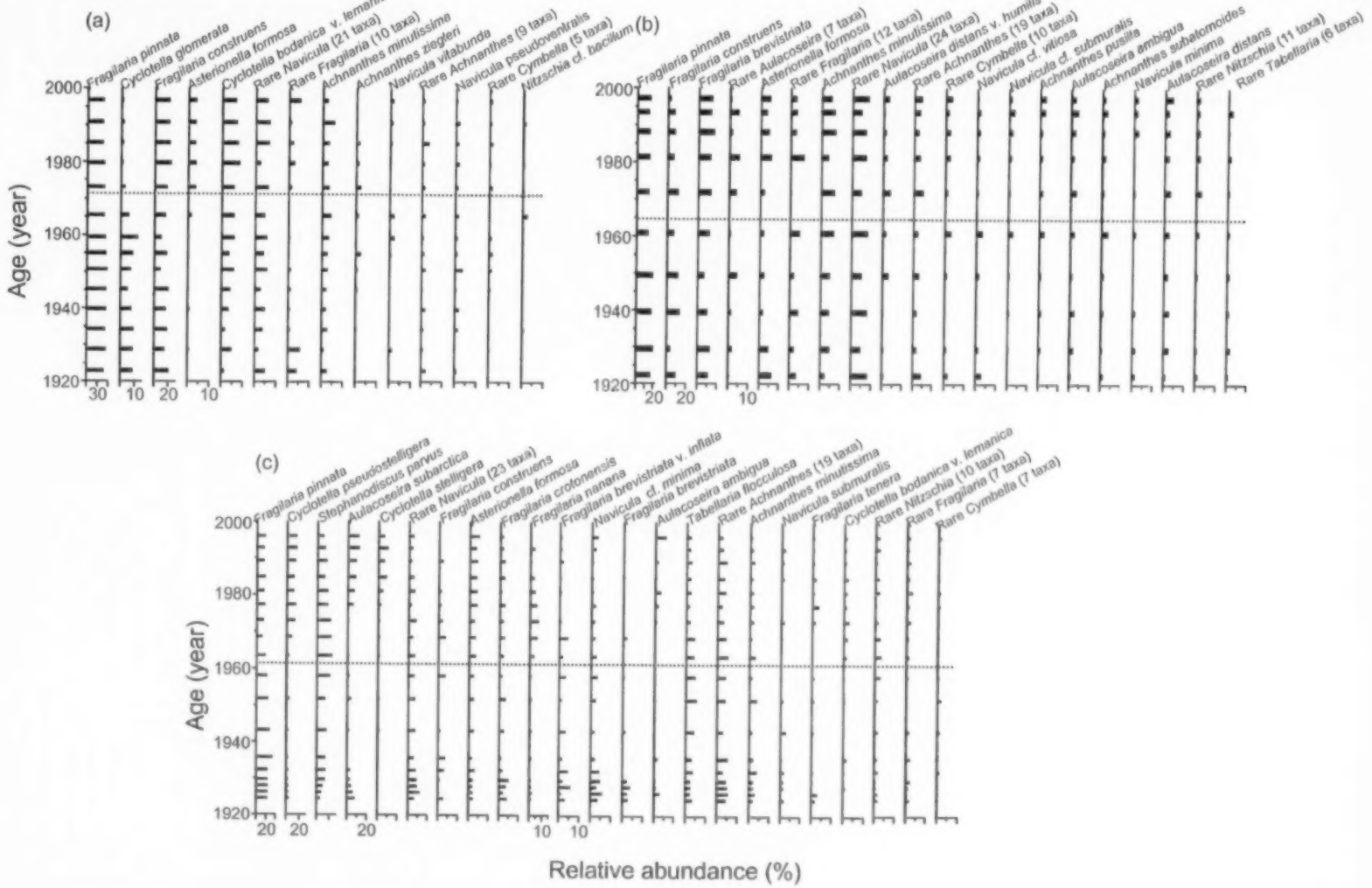
Fig. 5. Diatom taxa with frequencies greater than 2% in at least two samples for the low-cut impact lakes: (*a*) Woodcock, (*b*) Pitoney, and (*c*) Laurie. Taxa are ordered according to their contribution to the average dissimilarity (SIMPER score) between the two groups (before and after the onset of logging, denoted by the dotted line). Note the different percentage scales for the various taxa.



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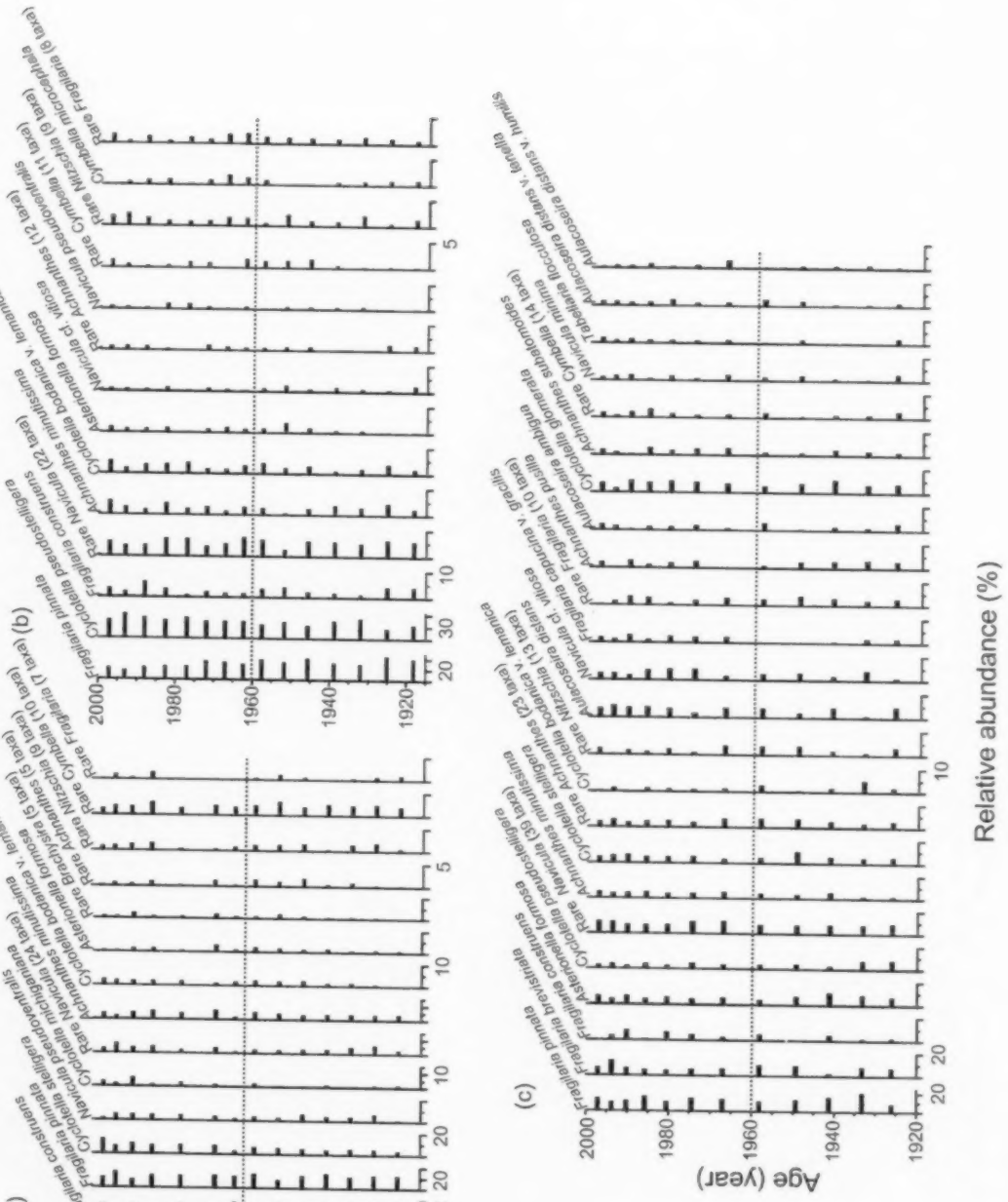

Fig. 6. Diatom taxa with frequencies greater than 2% in at least two samples for the high-cut impact lakes: (*a*) Upper Summit, (*b*) Jakes, and (*c*) Tang. Taxa are ordered according to their contribution to the average dissimilarity (SIMPER score) between the two groups (before and after the onset of logging, denoted by the dotted line). Note the different percentage scales for the various taxa.

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Table 3. Results of analysis of similarity (ANOSIM).

	Reference lakes				Impact lakes					
					Low cut			High cut		
	Boomerang	Justine	Secord	Unnamed	Laurie	Pitóney	Woodcock	Jakes	Tang	Upper Summit
Before and after 1960										
n (before/after)	4/6	7/8	5/9	8/8	9/9	5/5		7/8	5/7	7/7
R	0.278	0.115	0.211	0.053	0.819	0.344		0.289	0.250	0.116
p	0.048*	0.070	0.066	0.234	0.001**	0.056		0.015*	0.030*	0.119
Before and after 1975										
n (before/after)	6/4	10/5	8/6	11/5			9/5			
R	0.036	0.477	0.231	0.146			0.785			
p	0.376	0.001**	0.030*	0.106			0.001**			

Note: Number of samples (*n*) is indicated for before and after the reference year. *, $p < 0.05$; **, $p < 0.01$.

were low. Other fairly diverse genera in many of the lakes include *Cymbella* Agardh, *Nitzschia* Hassall, and *Fragilaria* Lyngbye.

Reference lakes

Three of the four reference lakes (Boomerang, Justine, and Secord) had a significant increase in percent organic matter in the sediment cores after 1960 (Table 2). However, the increases in percent organic matter were quite small, ranging from 2% in Boomerang Lake up to approximately 5% in Secord Lake (Figs. 3*a*–3*c*). The percent organic matter was quite variable in Unnamed Lake (Fig. 3*d*), but changes before and after 1960 were not significant.

The null hypothesis of no difference between diatom assemblages before and after 1960 or before and after 1975 could not be accepted in three of the four reference lakes (Table 3). Boomerang Lake showed a significant difference in species assemblages before and after ca. 1960, whereas Secord and Justine lakes showed significant differences in species assemblages before and after ca. 1975 (Table 3). In Boomerang Lake, the diatom species changes were quite small. Taxa such as *F. pinnata* and *A. formosa* decrease by approximately 3%, whereas *C. glomerata* increases by approximately 4% (Fig. 4*a*). In Justine Lake, there was a distinct, although small (4%), increase in *A. formosa* at ca. 1980 and an approximately 6% decrease in *F. pinnata* after 1975 (Fig. 4*b*). In Secord Lake, the changes in diatom species composition were subtle, with changes varying from <1% up to 3% for individual taxa (Fig. 4*c*).

Low-cut lakes

Only one of the low-cut impact lakes (Woodcock) showed a significant change in organic matter after the onset of logging (Table 2). Although the increase in mean percent organic matter in Woodcock Lake was small (~2%), the change is consistently higher with little variability around the mean either before or after 1975 (Fig. 3*g*). Although results were insignificant in Laurie Lake before and after 1960, there was a distinct increase of approximately 8% in organic matter starting at ca. 1980 (Fig. 3*e*). In Pitoney Lake, there was a distinct, although small (2%), decrease in organic matter between 1966 and 1976, which corresponds to a majority of the forest-harvesting activities in this basin (Fig. 3*f*).

Two of the low-cut impact lakes (Laurie and Woodcock) showed a significant difference in diatom species composition before and after the onset of logging and are associated with a high *R* statistic (Table 3). In Woodcock Lake, there was a distinct 6% decrease in *C. glomerata*, a 7% decrease in *F. pinnata*, and a distinct, although small (3%), increase in *A. formosa* with the onset of logging (Fig. 5*a*). In Laurie Lake, there were distinct increases in taxa such as *C. pseudostelligera* (8%) and *Fragilaria nanana* Lange-Bertalot (2%) with the onset of logging, and increases in *A. subarctica* (2%) and *Cyclotella stelligera* (Cleve and Grun. in Cleve) Van Heurck (4%) at ca. 1975 (Fig. 5*c*). *Fragilaria pinnata*, on the other hand, decreased by 10% with the onset of logging, whereas *Stephanodiscus parvus* Stoermer and Håkansson initially increased and then decreased to prelogging abundances by ca. 1975 (Fig. 5*c*).

High-cut lakes

No high-cut impact lakes showed a significant difference in percent organic matter before and after the onset of logging (Table 2). However, in Tang Lake, there was a slight decrease in organic matter after the onset of logging (Fig. 3*i*), with the significance of this change being just above the 0.05 level (Table 2). As well, in Jakes Lake, there was a distinct but small decrease in organic matter corresponding to the onset of logging in the watershed (Fig. 3*h*), whereas in Upper Summit Lake, the percentage of organic matter has changed very little over the last 80 years (Fig. 3*j*).

Two of the high-cut impact lakes (Jakes and Tang) showed a significant difference in diatom species composition before and after the onset of logging (Table 3). The changes seen in these two lakes were more subtle than those seen in the low-cut impact lakes Laurie and Woodcock, as indicated by their much lower *R* statistics. There is a higher abundance of rare taxa contributing to the difference between before and after logging and, thus, the significant changes in Jakes and Tang lakes are, in part, the result of minor differences in the mean abundance of many taxa. In Jakes Lake, *F. pinnata* decreased by 11% and *C. pseudostelligera* increased by 10% after logging (Fig. 6*b*), whereas all other taxa changed by less than 1%. In Tang Lake, taxa such as *F. pinnata* and *Fragilaria brevistriata* Grunow in Van Heurck are, on average, less abundant after the onset of logging, whereas *Fragilaria construens* (Ehrenberg) Grunow is, on average, slightly more abundant (Fig. 6*c*).

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In general, the variations in diatom species abundances in the lakes that showed significant differences in our ANOSIM analyses (three reference lakes: Boomerang, Justine, and Secord; two low-cut impact lakes: Laurie and Woodcock; two high-cut impact lakes: Jakes and Tang) were quite small, with changes in the relative abundance values being maximally ~10% (Figs. 4, 5, and 6). However, the variations in diatom community composition are more distinct in lakes that had an associated high *R* value (Laurie and Woodcock lakes; Table 3).

Ordination results of the physical characteristics of the lakes

A PCA of the physical characteristics of the lakes (not shown) did not reveal any striking differences between the impact and reference lakes. There is no evidence from the PCA analysis that the lakes which showed the greatest changes in diatom species composition (Laurie and Woodcock) have physical characteristics or linear combinations of the measured physical characteristics that would make them more sensitive to forestry impacts. This is further supported by canonical variates analysis of two groups of lakes—those with a significant response of diatoms and a high *R* statistic (Laurie and Woodcock lakes) and those with a low *R* statistic). The difference in the two groups could not be explained by any of the physical characteristics of the lakes or their watersheds. This may be due, in part, to the small number of lakes examined and the limited number of physical variables included in the analysis.

Discussion

Both natural and anthropogenic disturbances can influence terrestrial and aquatic ecosystems. The before–after design of this study in both impact and reference lakes from the same biogeoclimatic setting is a means of evaluating changes that are due to forestry activities in the context of changes that are due to natural factors (e.g., climate, fires, etc.). A similar design was used to assess the impact of logging and fires on lakes in northwestern Ontario (Paterson et al. 1998). Paterson et al. (1998) observed changes in chrysophyte assemblages in both the impact and reference lakes, and concluded that the observed changes were probably the result of a regional drought in the area that likely influenced the impact of forestry and fire on lakes in this region. In our study, the degree of change in the diatom assemblages in three of the five impact lakes with logging beginning in ca. 1960 (Laurie, Jakes, and Tang) was, on average, greater than in the reference lakes; however, in one of the reference lakes (Boomerang), there was as great a change as in two of the impact lakes (Jakes and Tang). Analysis of the diatom assemblages in Woodcock Lake, in which logging began in ca. 1975, indicated significant changes in species composition before and after the onset of logging. However, significant changes were also detected before and after 1975 in two of the reference lakes (Justine and Secord). The organic-matter content of the lake sediments showed a significant increase after 1960 in three of the four reference lakes (Boomerang, Justine, and Secord) but in only one of the impact lakes (Woodcock).

Increases in organic matter can be attributed to several factors, including increased in-lake production of organic matter, increased inwash of terrestrial-based organic matter, or decreases in the load of inorganic matter to the lake. Increased erosion and increased inputs of minerogenic sediments to lake basins have been shown to occur in some watersheds that have experienced fire (MacDonald et al. 1991; Rhodes and Davis 1995). However, fires of limited spatial extent and intensity may not be detected in the lake-sediment record (MacDonald et al. 1991). Fires likely occurred within the watersheds of some of our reference lakes prior to 1960, as determined from the age structure of the forests (see Study area, sites, and study design, above). However, it is unlikely that the significant changes seen in the organic profiles of the three reference lakes are associated with these fires, because the changes observed in the reference lakes are directional increases in organics that have occurred over the last two to three decades. Catchment disturbance by fire would typically result in a decreased concentration of organic matter in lake sediments, because fires are normally associated with periods of increased erosion of inorganic matter that are typically of relatively short duration (Beaty 1994; Rhodes and Davis 1995). Another hypothesis for a significant increase in organics is that the processes of nutrient cycling in the younger forest are quite different from those in the predisturbance forest and, thus, the changes seen in the reference lakes may be due to natural recovery and aggradation processes (e.g., Bormann and Likens 1979). This, however, seems unlikely, because the forest stands in many of the reference lakes are of different age-classes. For example, Unnamed Lake, with a watershed in which nearly 50% of the stands are less than 100 years of age (Spicer 1999), did not show any significant change in organic matter or diatoms, whereas Boomerang Lake, with a watershed in which only approximately 6% of the stands are less than 100 years of age (Spicer 1999), showed significant changes in both diatom species composition and percent organic matter. However, Unnamed Lake has no inflowing surface streams and, thus, terrestrial inputs from runoff may be minimal in comparison with the other lakes. Of the reference lakes, only Boomerang Lake showed significant changes, albeit subtle, in diatom assemblage before and after 1960. Although there were significant changes in the organic matter before and after 1960 in the reference lakes Justine and Secord, these changes did not translate into distinct changes in the diatom flora at that time that could be related to increased nutrients or other chemical changes. Although extensive fire or some other widespread disturbance (e.g., windthrow, insect infestation) occurred in the reference watersheds of Justine and Secord lakes prior to 1960, it apparently had a minimal effect on the diatom assemblages of these lakes. Another potential influence on the organic-matter profiles seen in the sediment cores of the reference lakes (Boomerang, Justine, and Secord) is diagenesis of the sediment organic matter, which could result in decreased organic matter downcore (e.g., Carignan and Lean 1991).

Based on the results of the ordination analyses, there were no apparent unique physical characteristics (or combination of measured characteristics) that would make Laurie and Woodcock lakes, which showed the greatest changes in diatom species composition coincident with the onset of log-

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ging, more sensitive to logging activities than the other lakes. The diatom changes seen in Woodcock Lake after the onset of logging in 1975 may be related to increased phosphorus inputs, with a decrease in the planktonic oligotrophic taxon *C. glomerata* and an increase in the planktonic mesotrophic taxon *A. formosa*. This observation is consistent with the significant increase in organic content of the sediment record at this time. A similar increase in *A. formosa* was observed in Justine Lake (reference) at ca. 1980, which also corresponds to an increase in organic matter. Laurie Lake has been a fairly productive lake during at least the past 80 years, with taxa characteristic of meso- to eu-trophic conditions, such as *S. parvus*, *A. formosa*, and *F. crotonensis*, being present throughout the core. Curiously, the oligotrophic taxon *C. pseudostelligera* increases with the onset of logging in 1964, along with a slight increase in *S. parvus*. At ca. 1975, there are increases in *C. stelligera*, another oligotrophic taxon, as well as in the eutrophic planktonic taxa *A. subarctica* and *Aulacoseira ambigua* (Grun. in Van Heurck) Simonsen. Increases in these taxa correspond to increased organic matter in the sediments, suggesting an overall increase in productivity within this lake.

Jakes and Upper Summit lakes showed no or minimal changes in diatom species composition or percent organic matter despite the harvesting of over 60% of their watersheds (a harvest much larger than that in Laurie and Woodcock lakes). While a high density of roads in the basins of Upper Summit and Jakes lakes (Spicer 1999) would help to promote sediment inputs into these basins, the watersheds of these lakes have a relatively small number of streams, which could limit sediment input. As well, that no or minimal changes in diatom species composition and percent organic matter were observed in Pitoney and Tang lakes may be the result of upstream sediment storage, since these two basins contain a greater number of upstream lakes and ponds in comparison with the other lakes examined (Spicer 1999).

Carignan et al. (2000) proposed an intuitively simple model to explain the degree of impact on water quality from forest harvesting on lakes that is based on the area of watershed cut and lake area. According to this model, the greater the ratio of area harvested divided by either the lake's volume or its surface area, the greater the impact. Applying this model to our study lakes, the greatest impact should have been seen in Pitoney and Tang lakes. The Carignan et al. (2000) model may not be applicable to our study region, or to the Vancouver Island study region (Laird et al. 2001, this issue), owing to the different physiographic and edaphic conditions and the different time frames of the studies. The differences in our findings may also be due to uncertainties in their model related to deriving preharvest conditions on the water chemistry of the logged lakes from a set of reference lakes.

It is difficult to decipher whether the significant diatom changes we see in four of the six impact lakes (two low cut, two high cut) are due solely to forest harvesting, because significant changes were also observed in three of the reference lakes. Furthermore, there were significant changes in the profiles of organic matter of the same three reference lakes (Boomerang, Justine, and Secord). There are no other known human disturbances (e.g., cottage development, mining, fish stocking, etc.) within the reference watersheds that could account for these changes. Natural watershed processes associated with fire and younger-age stands may have had an affect on the reference lakes; however, the pattern of percentage of stands aged <100 years and the associated diatom and organic-matter responses, as outlined earlier, are not consistent across the basins. Climatic records from Prince George, B.C., indicate some trends in annual temperature and precipitation since the mid-1970s; temperature and annual precipitation have typically been above and below the 50-year average, respectively. These changes in climate may be influencing some of the trends seen in the organic matter and diatoms, however again the timing and magnitude of the changes are not consistent across basins.

Regardless of whether all the changes seen in the impact lakes are due solely to logging or are confounded by natural processes, the changes are minimal in comparison with what has been documented for other land-use changes. Intensive land-use changes associated with permanent land clearance for agriculture and settlement have had dramatic effects on diatom floras compared with the impacts seen in our study where the forests were allowed to regrow. For example, early land clearance associated with agriculture during the Neolithic and Bronze Ages in the fertile lowlands of East Anglia in Norfolk, U.K., resulted in distinct changes in the diatom flora and pigments that were suggestive of nutrient enrichment of Diss Mere Lake (Fritz 1989). Subsequent widespread land clearance and intensive land use resulted in the eutrophication of this lake. Increased arable agriculture and settlement around the mere helped to maintain the high nutrient levels within it. In northern Sweden, initial land clearance and later continuous arable farming resulted in distinct changes in the diatom flora that were suggestive of the nutrient enrichment within Kassjön Lake (Anderson et al. 1995). Renberg et al. (1993) found an increase in diatom-inferred pH in 14 lakes in Sweden that was the result of intensive land-use changes associated with burning, agriculture and forest grazing. The distinct changes seen in these studies are likely due, in large part, to the severity and duration of hundreds to thousands of years of land clearance. Deforestation, as a result of logging and farming, with subsequent urbanization, has also been shown to have a large impact on lakes (e.g., Brugam and Vallarino 1989). In Brugam and Vallarino's (1989) study of four lakes in western Washington, U.S.A., initial deforestation due to logging and farming resulted in changes in diatom composition suggestive of increased conductivity and pH. However, the subsequent urbanization of these watersheds resulted in the largest changes in diatom species composition, which are suggestive of nutrient enrichment. Such intensive and continued land use, as outlined in the above studies, would result in sustained changes in the hydrological, biological, and soil processes that link the terrestrial and aquatic ecosystems. Other factors that can influence the response of a lake to land-use changes include climate, watershed characteristics (e.g., size, slopes, streams, vegetation type), and lake basin characteristics (e.g., surface area, depth, water residence time), as well as the mineralogy and nutrient status of the catchment soils. For example, on richer soils in Michigan, diatom changes suggest increased nutrient levels associated with land clearance for logging and subsequent settlement (Fritz et al. 1993). In the Pocono Mountains of Pennsylvania, Lott et al.

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(1994) found that distinct changes in scaled chrysophytes occurred when the watershed was heavily logged, presumably due, in part, to richer soils. Paterson et al. (1998), however, saw minimal changes in scaled chrysophytes associated with logging in lakes in northwestern Ontario on the Canadian Shield, which has shallow poor soils and abundant wetlands that may have large storage capacities. In the central interior region of British Columbia (this study), on the west coast of Vancouver Island (Laird et al. 2001, this issue), and in northwestern Ontario (Paterson et al. 1998), the minimal changes to lake systems following forest harvesting may, in part, be due to the rapid regrowth of these areas. Rapid revegetation would result in relatively short-term alterations to the terrestrial–aquatic links and cause no prolonged changes in water chemistry. This is in contrast with the large changes in algal species observed in studies of lake acidification (e.g., Cumming et al. 1994), eutrophication (e.g., reviewed in Hall and Smol 1999), and climate change (e.g., Laird et al. 1996).

Other possible reasons for the apparent minimal effects of logging on these central interior B.C. lakes may be similar to those discussed in Laird et al. (2001, this issue), and include: (*i*) changes in water chemistry are short lived and, therefore, not detected at the study resolution of 5–10 years; and (*ii*) the higher pH and alkalinity of these lakes may provide a greater buffering capacity compared with lakes in other studies, in which pH changes were shown to be associated with logging. Another reason given for the minimal influence of logging on the lakes on Vancouver Island (Laird et al. 2001, this issue), was the low percentage of watershed cut in any particular year. However, in this study, Jakes and Upper Summit lakes had 50–60% of the watershed logged in a single year and showed only minor changes in diatom species abundances.

In the Vancouver Island study (Laird et al. 2001, this issue), all the impact lakes showed a significant, although small, change in diatom species composition, whereas only one reference lake showed significant changes. In their study, Laird et al. (2001, this issue) suggest that the significant diatom response of this one reference lake may be indicative of a local watershed disturbance, since the other reference lakes showed no significant changes. The more dilute oligotrophic lakes of Vancouver Island, with their steeper slopes compared with the central interior, may be more sensitive to logging activities. However, in lakes in the central interior, confounding influences of the different-age stands in the reference lakes, and the potential influence of climate make it difficult to assess the changes that are solely due to forest harvesting. Regardless, in both study regions in British Columbia—even in those lakes that showed significant changes in diatom species composition—the changes were typically small shifts in percent abundance as opposed to the appearance or disappearance of diatom taxa.

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Effects of anoxia and sulfide on concentrations of total and methyl mercury in sediment and water in two Hg-polluted lakes

Olof Regnell, Tommy Hammar, Anders Helgée, and Bo Troedsson

Abstract: Between May and December 1996, monthly samples of surface sediment (0–1 cm), settling matter, and water were taken at a shallow site and a deep site in each of two consecutive Hg-polluted riverine lakes. In the upper lake, the sediment was polluted also with cellulose fiber. Both hypolimnia turned anoxic, but sulfide was detected only in the upper lake. When sulfide appeared, hypolimnetic methyl mercury (MeHg) increased and reached 47 pM (9.4 ng·L^{-1}), whereas MeHg in the sediment below decreased. The increase in hypolimnetic inorganic Hg (IHg = total Hg – MeHg), which reached a peak of 40 pM (8.0 ng·L^{-1}), was slower, possibly because mobilized IHg was methylated. In the lower lake, hypolimnetic MeHg and IHg increased less dramatically during summer stratification, reaching only 5 and 24 pM (1.0 and 4.8 ng·L^{-1}), respectively. There was no detectable concomitant decrease in sediment MeHg. In both lakes, MeHg appeared to increase simultaneously with total Fe and Mn in the hypolimnion, as did IHg in the lower lake. Our observations suggest that the presence of hydrous ferric and manganese oxides decreased the mobility of Hg in both lakes but increased MeHg production in the upper lake.

Résumé : De mai à décembre 1996, nous avons prélevé à chaque mois des sédiments de surface (0–1 cm), de la matière en sédimentation et de l'eau à un site de faible profondeur et à un site plus profond dans chacun de deux lacs fluviaux situés en enfilade et pollués au Hg. Les sédiments du lac d'amont étaient aussi contaminés par des fibres de cellulose. Les hypolimnions des deux lacs sont devenus anoxiques, mais le sulfure n'a été détecté que dans le lac d'amont. À l'apparition du sulfure, le méthyl-mercure (MeHg) s'est accru et a atteint 47 pM (9,4 ng·L^{-1}) dans l'hypolimnion, alors qu'il a diminué dans les sédiments sous-jacents. L'accroissement du Hg inorganique (IHg = Hg total – MeHg) dans l'hypolimnion s'est fait plus lentement avec un maximum à 40 pM (8,0 ng·L^{-1}), probablement parce que le Hg libéré a subi la méthylation. Dans le lac d'aval, le MeHg et l'IHg de l'hypolimnion ont crû de façon moins spectaculaire pendant la stratification d'été, atteignant respectivement seulement 5 pM (1,0 ng·L^{-1}) et 24 pM (4,8 ng·L^{-1}); il n'y a pas eu de chute concomitante du MeHg dans les sédiments. Dans les deux lacs, l'accroissement du MeHg s'est accompagné d'une augmentation du Fe total et du Mn dans l'hypolimnion; il en a été de même dans le cas du IHg du lac d'aval. Nos observations laissent croire que la présence d'oxyde ferrique hydraté et d'oxyde de manganèse a diminué la mobilité du Hg dans les deux lacs, mais qu'elle a augmenté la production de MeHg dans le lac supérieur.

[Traduit par la Rédaction]

Introduction

Mercury is a nonessential heavy metal that is highly toxic to all organisms. Its monomethylated form, methyl mercury (MeHg), is a human health concern and poses a threat mainly to those with a high intake of piscivorous freshwater fish. MeHg, as opposed to inorganic Hg (IHg = total Hg – MeHg), is highly bioaccumulated in aquatic food chains and may reach concentrations in fish six to seven orders of magnitude higher than in water (Driscoll et al. 1994).

Mercury is transported in rivers in both dissolved and particulate form, the particulate fraction increasing during high flow (Jackson et al. 1982). When a river passes through a lake, a major part of the particle-bound Hg may settle to the bottom, whereas dissolved forms may be scavenged by seston, notably phytoplankton (Kirkwood et al. 1999), and thereafter settle to the bottom. Dissolved Hg is also adsorbed directly by bottom sediment, volatilizes, or is simply transported out of the lake with outflowing water.

Several processes interfere with Hg accumulation in lake sediment. Release of Hg from particles will cause Hg to build up in the water and increase its export downstream. Another process of great concern is methylation of IHg to MeHg because it leads to a dramatic increase in bioavailability. Methylation may also increase the mobility of Hg in anaerobic environments (Regnell et al. 1996; Bloom et al. 1999). Reduction to Hg(0) and volatilization has been shown to significantly reduce the net input of Hg to lakes (e.g., Poissant et al. 2000).

Lake stratification is known to increase net MeHg produc-

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tion and also to increase the recycling of Hg in the water column and at the sediment–water interface. Typically, anoxic conditions develop in hypolimnia of stratified lakes, which may or may not be accompanied by increased levels of free sulfide (H_2S and HS^-). It is widely recognized that MeHg is produced mainly under anoxic conditions and that the major Hg methylators are found within the group of sulfate-reducing bacteria (Compeau and Bartha 1985). However, sulfide generated by these bacteria is known to inhibit Hg methylation by rendering Hg less available for methylation (Berman and Bartha 1986; Benoit et al. 1999).

Hypolimnetic enrichment of total Hg (THg) and MeHg is a common phenomenon and several mechanisms explaining it have been suggested: (*i*) reductive dissolution of iron and manganese oxides with which Hg(II) and MeHg are associated (e.g., Cossa et al. 1994; Jackson 1998), (*ii*) formation of water-soluble Hg(II)–sulfide complexes and MeHgSH (e.g., Wang and Driscoll 1995; Regnell et al. 1997), (*iii*) mineralization of settling biotic particles, leading to dissolved or colloidal forms of Hg (Hurley et al. 1991), (*iv*) sedimentation of dense layers of seston that have accumulated Hg in the upper water layers (Hurley et al. 1991), and (*v*) in situ methylation of IHg (Watras et al. 1995*a*).

The present study was designed primarily to shed light on the effect of stratification, anoxia, and sulfide on retention and recycling of THg and MeHg in two consecutive Hg-polluted boreal lakes in a Swedish river system. In order to meet this goal, we followed seasonal variation in the levels of THg, MeHg, and various physicochemical variables in the water, settling matter, and surface sediment from May to December in 1996.

Materials and methods

Lakes

The study lakes, Övre Svartsjön and Nedre Svartsjön (referred to below as "the upper lake" and "the lower lake") are situated in southeastern Sweden. A small river (yearly average flow 0.9 $m^3 \cdot s^{-1}$) passes through the lakes, the lower lake being immediately downstream of the upper lake (Fig. 1). The theoretical residence time of the water, based on the average yearly flow, was 4 days in the upper lake and 10 days in the lower lake. Upstream from the lakes, a paper mill is situated that released cellulose fibers, mostly from unbleached ground wood pulp, into the river until new treatment facilities were constructed in the early 1970s. Between 1940 and 1965, the pulp was impregnated with phenyl mercury acetate to prevent slime growth. Because of the low stability of phenyl mercury (Jackson 1998), we thought that it was reasonable to assume that all phenyl mercury acetate had been converted to IHg and MeHg.

The upper lake, being the first major sedimentation basin, has accumulated large amounts of cellulose fibers. According to a recent survey, it contains approximately 14 400 t (dry) of sediment containing cellulose fibers showing variable degrees of degradation. In the lower lake, no cellulose fibers were found, but its bottom sediment may contain material originating from cellulose fiber. Basic limnological data for the two lakes are given in Table 1.

Sampling strategy

Water samples were collected monthly (May–December 1996) at the site of maximum depth in each lake. On all occasions, water was sampled from 0.5 m below the surface and 0.5 m above the bottom sediment. In July and August, water samples were collected from three additional depths in the thermocline. In the lower lake, which was stratified longer, this was done also in September.

Surface sediment samples (0–1 cm) were collected from two stations in each lake, one at a shallow site where the sediment–water interface was always well oxygenated and the other at the site of maximum depth. Four or five sediment samples were taken from each station on each occasion. Locations of the sampling sites are shown in Fig. 1. In the fall of 1999, one core was taken at each station in the lower lake in order to determine the vertical distribution of Hg and other constituents in the sediment.

Sediment traps (Limnos OY, Finland), inner diameter = 62 mm and height = 400 mm, were placed 1 m above the sediment–water interface in the vicinity of each of the four stations for sediment sampling. These were emptied monthly between June and December. No preservative was added to the traps.

Fig. 1. Lakes of the present study. The location of the sampling stations and depth isopleths (m) are shown. The arrows indicate the direction of water flow. The inset map shows the location of the study lakes.

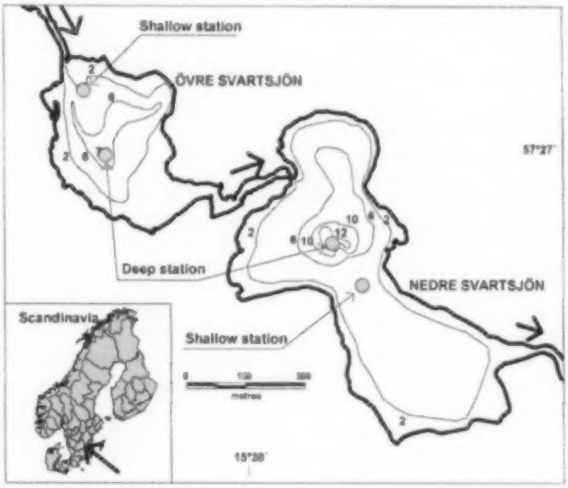

Table 1. Basic features of the two study lakes.

Variable	Upper lake	Lower lake
Area (km^2)	0.12	0.25
Mean depth (m)	2.4	3.2
Maximum depth (m)	7.2	12.1
pH	6.2–7.1	6.5–7.1
Alkalinity (mM)	0.18–0.36	0.19–0.36
Color (mg Pt·L^{-1})	20–100	15–100
Total N (μM)	20–41	19–38
Total P (μM)	0.13–0.45	0.13–0.45

Note: The concentration ranges are for epilimnetic water during 1996.

Sampling methods

Water for all types of analyses was collected using a metal-free Ruttner sampler, which was rinsed with ultraclean HNO_3 and MQ water before use. Teflon bottles (120 mL), filled with ultraclean MQ water and 0.5 mL of concentrated HCl, were emptied and rinsed twice with the sample water before being completely filled with sample. Each Teflon bottle was then double bagged using plastic polyethylene bags and placed in a refrigerated box. To

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avoid contamination, plastic gloves were used during sampling. The samples were immediately transported to the laboratory. There, prior to storage at 6–9°C, 0.6 mL of HCl was added to each sample.

Sediment samples were obtained with a corer of our own design equipped with plastic liners. Only the top 0–1 cm of the sediment was transferred to plastic jars. These were double bagged and placed in a refrigerated box and then stored frozen until analysis. The sediment trap material was handled in a similar manner.

Analytical procedures

THg in water was quantified using a double-amalgamation technique and atomic fluorescence detection. Sediment samples were wet oxidized in Teflon containers to which concentrated HNO_3 was added. The Teflon containers were placed in an autoclave (123°C) for 1 h. The samples were then transferred to a reaction vessel to which $SnCl_2$ was added to convert all Hg to elemental Hg. A cold-vapor atomic absorption spectrophotometer (LDC mercury monitor) was used for quantification. See Table 2 for detection limits and precision of measurements.

MeHg was determined by the method of Bloom (1989). However, instead of extraction, the initial cleanup and preconcentration step was distillation (Horvat et al. 1988). In order to quantify MeHg in sediment samples, all MeHg was transferred to water using a similar distillation procedure as for water samples. Artifact formation of MeHg during distillation has been demonstrated. This could be significant for sediment samples with <1% of THg as MeHg (Bloom et al. 1997). Since the proportion of THg present as MeHg in the majority of our sediment samples was well above 1%, we are confident that any artifact MeHg could be safely neglected. See Table 2 for detection limits and precision of measurements. A more thorough description of the analytical procedures for the determination of THg and MeHg in water and MeHg in sediment is given in Regnell et al. (1997).

Total Fe and Mn were determined in water samples that were acidified with HNO_3 using an ICP-ES instrument (Perkin Elmer, plasma II emission spectrometer). Total Fe, Mn, P, and S were quantified in lyophilized sediment samples after wet oxidation using the same instrument (see Regnell et al. 1997).

Total organic carbon (TOC) in water was determined using a carbon analyzer (Shimadzu, TOC-500). Particulate organic carbon (POC) was estimated from the concentration of suspended particulate matter (SPM), the ignition loss of SPM, and the relationship between ignition loss and total C in the surface sediment below (ignition loss = $K \times$ total C, where $K = 2.0$ and 2.1 in the upper and lower lake, respectively). Dissolved organic carbon (DOC) was calculated by subtraction. The total C content of the sediment was determined using a carbon analyzer for solid samples (LECO). Since both study lakes are softwater lakes (low alkalinities), it can be assumed that total C represented TOC in the sediment.

Sulfide in water samples was measured colorimetrically using the methylene blue method (Fonselius 1983). The samples were stabilized in the field by adding solutions of zinc acetate and NaOH. Water temperature and dissolved oxygen were measured directly in the field with a temperature–oxygen probe (YSI model 58).

Data analysis

To study covariation between two variables, Pearson's product-moment correlation coefficients (r) and associated significant probabilities (P) were computed for untransformed data (log transformation resulted in virtually the same values). For each correlation matrix, the P values of the correlation coefficients were corrected using a sequential Bonferroni test (Sokal and Rohlf 1995). When pairwise relationships between sediment variables were investigated, mean values from each sampling date ($n = 4$–5) were used. The correlation coefficients thus reflected temporal rather than small-scale spatial relationships between the variables at each station. To further illustrate covariation between sediment variables, principal components analysis (PCA) was performed. All variables were standardized prior to performing the PCA. Because all the individual sediment samples at one station were used in this calculation, the PCAs reflected not only temporal variation but also small-scale spatial variation at the four sampling stations. Spatial variation would result from the fact that samples were taken at least a few inches apart on the same sampling occasion and probably metres apart between sampling occasions.

Results and discussion

Enrichment of MeHg and IHg in the hypolimnetic water

In both lakes, [MeHg] and [IHg] increased in the anoxic hypolimnion during summer stratification. A constant rate of release from the bottom sediment and (or) settling matter does not conform to the fact that the lakes were stratified long before [MeHg] and [IHg] started to rise in the hypolimnion (cf. Figs. 2 and 3). Moreover, in both lakes, the buildup of MeHg in the surficial sediment preceded that in the overlying water. The fact that [total Fe] and [total Mn] peaked simultaneously with [MeHg] and [IHg] in the hypolimnetic water of both lakes indicated that release of Hg occurred mainly at low redox potentials (Fig. 3).

Hydrous ferric and manganese oxides (HFO and HMO) have high capacity for binding both MeHg and Hg(II) (Cossa et al. 1994; Gagnon and Fisher 1997). The increase in [total Fe] and [total Mn] in the hypolimnion was most likely the result of reductive dissolution of HFO and HMO occurring both in the sediment and on suspended particles in the hypolimnion. The simultaneous increase in hypolimnetic [IHg] and [MeHg] could be explained by release of Hg(II) and MeHg in conjunction with reductive dissolution of HFO and HMO.

In the hypolimnion of the upper lake, the increase in [IHg] started approximately a month later than the increase in [MeHg], [total Fe], and [total Mn] (Fig. 3). Possibly, almost all Hg released from particulate matter was methylated during the initial phase of anoxia in the upper lake, after which methylation was inhibited by high sulfide levels, allowing a significant buildup of IHg. In the lower lake, in which methylation rates were much lower, [IHg] increased simultaneously with [MeHg] (Fig. 3).

In both lakes, the correlation between [MeHg] and [total Fe] in the hypolimnetic water was strong and highly significant statistically (z test, Bonferroni correction: $P < 0.01$), whereas that between [IHg] and [total Fe] was not (Table 3). This indicated that the redox cycle of Fe had a stronger influence on [MeHg] than on [IHg]. Bloom et al. (1999) found the highest [MeHg] in marine sediment pore water at the point of maximum [total Fe], whereas the solubility of IHg was unrelated to changes in [total Fe] and [total Mn]. They concluded that the mobility of MeHg was controlled by metal oxide solubilization and that the mobility of IHg was controlled by formation of soluble sulfide or organic complexes. We believe that, in addition to the direct association of MeHg with HFO, a positive relationship between [total Fe] and [MeHg] in the hypolimnetic water could be explained by the fact that reductive dissolution of HFO and production of MeHg are both the result of anaerobic microbial activity. Furthermore, both the solubility of Fe(II) and the production of MeHg are reduced by high sulfide levels.

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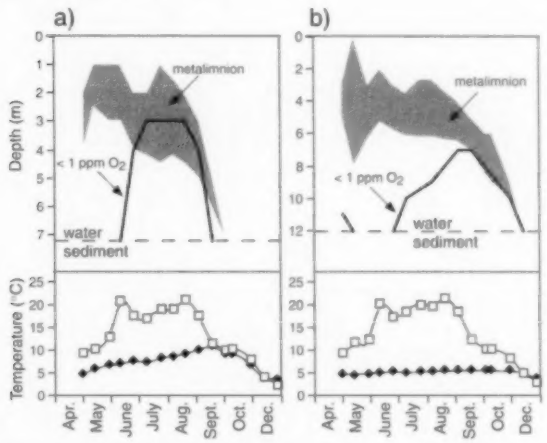
Table 2. Data on the analytical quality of the THg and MeHg measurements in the present study.

Detection limit[a]			
Water (pM)		Sediment (pmol·g dry^{-1})	
THg	MeHg	THg	MeHg
0.25	0.1	10	0.05

	Precision			
	THg (µg Hg·g dry^{-1})		MeHg (ng Hg·g dry^{-1})	
Reference material	Certified	Obtained	Certified	Obtained
IAEA-356 (marine sediment)			5.46±0.38	5.60±0.36[b]
CRM 320, BCR (river sediment)	1.03±0.13	1.02±0.07[c]		

[a]Valid for water samples of 30 and 50 mL (for MeHg and THg determinations, respectively) and for 1 g of wet sediment.
[b]n = 5.
[c]n = 12.

Fig. 2. Temporal variation in the extension of the metalimnion (thermocline), temperature in the surface and bottom water, and depth below which oxygen was <1 ppm during 1996 in the (*a*) upper lake and (*b*) lower lake. □, 0.5 m below the surface; ◆, at maximal water depth.



Sulfate-reducing bacteria, known to produce MeHg when free sulfide levels are low to moderate (Compeau and Bartha 1985, 1987), inhibit Hg methylation as well as remove Fe(II) from solution (FeS(s) formation) as a result of their production of sulfide.

For the upper lake, it is clear that the hypolimnetic buildup of Mn started earlier than that of MeHg (Figs. 3*a* and 3*e*), but this should not be taken as evidence that HMO is a poor scavenger of MeHg. While Mn(II) is known to be able to diffuse through oxic sediment layers (Davison et al. 1982), it seems probable that any MeHg released in conjunction with reductive dissolution of HMO would become trapped by oxides still remaining at the sediment–water interface. In addition, since most of the hypolimnetic MeHg probably was a result of in situ production rather than of release of MeHg from the sediment (see below), reductive dissolution of manganese oxide seemed to have started at higher redox potentials than Hg methylation.

Besides scavenging oxides, organic particles as well as dissolved organic complexing agents will affect the mobility of Hg. In the hypolimnion of the lower lake, in which sulfide could not be detected during the study period, [IHg] and [MeHg] peaked simultaneously with [DOC] and showed seasonal variation similar to that of [DOC] also for the rest of the study period (Figs. 3 and 4; Table 3). [DOC], in turn, peaked simultaneously with and showed a seasonal variation similar to that of [total Fe] and [total Mn] (Figs. 3 and 4). These results are in line with the earlier stated assumption that Hg(II) and MeHg are bound to HFO and HMO via organic ligands (Regnell et al. 1997). This is likely the case because virtually all filter-passing Hg(II) and MeHg in nonsulfidic water is bound to dissolved or colloidal organic matter (Stordal et al. 1996; Hintelmann et al. 1997) and because oxides are efficient scavengers of dissolved and colloidal organic compounds (Tipping and Woof 1983). However, as discussed by Tipping and Woof (1983), organic matter can accumulate in hypolimnia not only as a result of dissolution of oxides but also as a result of anaerobic decomposition of particulate organic matter resulting in dissolved and colloidal forms. A simultaneous buildup of total Fe could be explained by microbially mediated reduction of Fe(III) to more soluble Fe(II). Similarly, rather than being a result of dissolution of HFO and HMO, the hypolimnetic buildup of Hg could have been the result of decomposition of particulate organic matter to which it was bound. In line with this interpretation of the simultaneous buildup of THg, total Fe, and total Mn, Hurley et al. (1991) concluded that mineralization of settling particles of biological origin explained the hypolimnetic enrichment of THg in a Wisconsin seepage lake. Not knowing the composition of the particles carrying most of the Hg, we cannot tell whether organic matter decomposition was the primary cause of the hypolimnetic Hg enrichment or not. In any case, we believe that the redox cycles of Fe and Mn had a major effect on the amount of dissolved and colloidal Hg because any such Hg formed during organic matter degradation could be scavenged by HFO and HMO.

Even if not mineralized, dense layers of settling matter that have accumulated Hg, e.g., decaying phytoplankton, could give rise to what would appear as a hypolimnetic THg enrichment of short duration before ending up in the sediment. For our study lakes, a hypolimnetic enrichment of THg due high concentrations of settling (or resuspended) particles with which Hg was associated is not in line with our observation that [POC] and [SPM] did not increase simultaneously with [THg] (Fig. 4).

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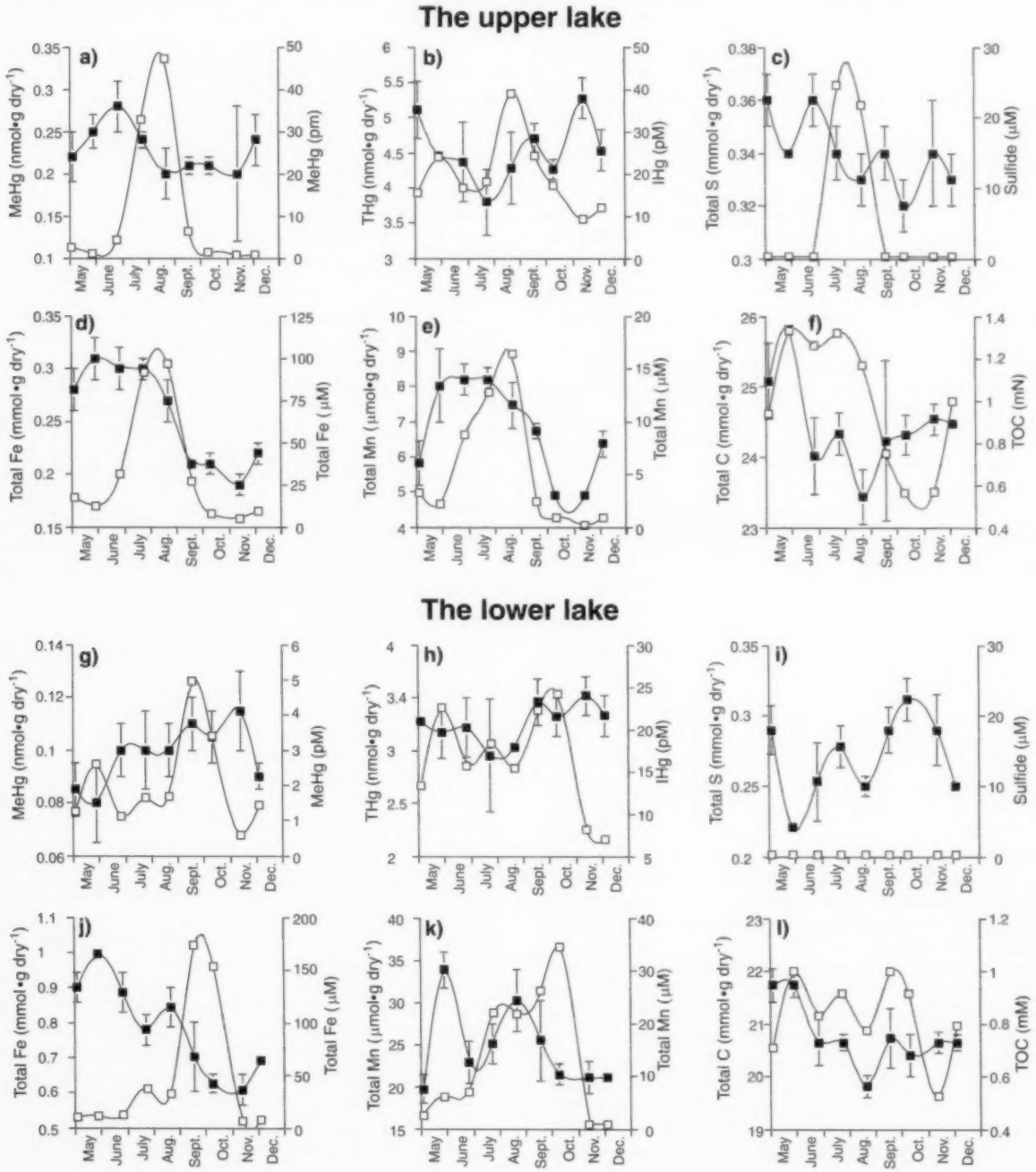
Fig. 3. Temporal variation in [MeHg], [THg], [IHg], and concentrations of ancillary constituents in the surface sediment (0–1 cm) and hypolimnetic water during 1996 in the (*a–f*) upper lake and (*g–l*) lower lake. The samples were taken at the deep stations. Bars represent ±1 SD (n = 4 or 5). The values for water are based on single samples. ■, sediment (0–1 cm); □, water (0.5 m above the sediment).



Yet another explanation for hypolimnetic Hg enrichment is that Hg(II) and MeHg form soluble complexes with bisulfide and (or) polysulfide or that Hg(II) forms colloidal HgS (Wang and Driscoll 1995; Hintelmann et al. 1997; Paquette and Helz 1997). However, precipitation of Hg(II) as HgS(s) or its coprecipitation with FeS may also occur, depending on several factors such as the free sulfide concentration, pH, Fe(II) concentration, and the level and quality of dissolved organic matter (Dyrssen and Wedborg 1991; Ravichandran et al. 1999). The fact that sulfide levels were high

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Fig. 4. Temporal variation in [THg], [DOC], [POC], and [SPM] in the hypolimnetic water during 1996 in the (*a*) upper lake and (*b*) lower lake. The values are based on single samples.

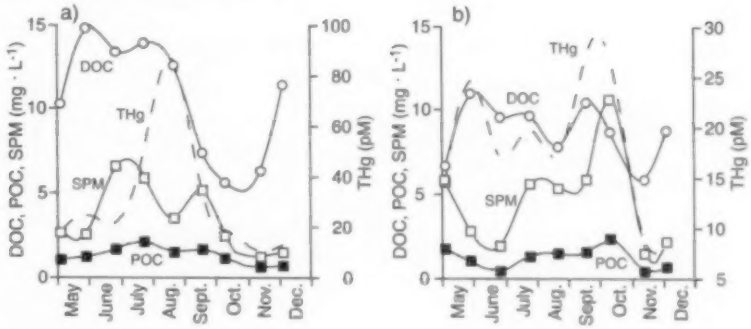

Table 3. Pairwise correlation (*r*) with the variables indicated.

Variables	Upper lake	Lower lake
MeHg vs. TOC	0.45	0.75
MeHg vs. POC	0.58	0.56
MeHg vs. DOC	0.40	0.60
MeHg vs. SPM	0.36	0.56
MeHg vs. total Fe	0.97***	0.90**
MeHg vs. total Mn	0.92***	0.69
MeHg vs. IHg	0.73	0.76
IHg vs. TOC	0.37	0.82*
IHg vs. POC	0.48	0.62
IHg vs. DOC	0.32	0.66
IHg vs. SPM	0.26	0.64
IHg vs. total Fe	0.65	0.68
IHg vs. total Mn	0.66	0.74

Note: These were measured on nine occasions at approximately monthly intervals between May and December 1996 in the hypolimnetic water of the upper lake and lower lake.*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

when [IHg] and [MeHg] peaked in the hypolimnion of the upper lake indicates that the solubility of Hg increased instead of decreasing in the presence of sulfide (Fig. 3). Furthermore, the lack of a clear relationship between Hg and DOC (Figs. 3 and 4; Table 3) suggests that Hg formed complexes with sulfide rather than with DOC during the buildup of Hg in the hypolimnion. The sulfide levels that we measured in the hypolimnion of the upper lake fell within the range that Benoit et al. (1999) stated would lead to sulfide complexation of Hg(II) and to negligible amounts of Hg(II)–DOC complexes. Also, using the equilibrium calculations provided by Hintelmann et al. (1997), sulfide would outcompete DOC in the complexation of MeHg.

High [THg] in sulfidic hypolimnetic water has been observed by others (e.g., Wang and Driscoll 1995). Furthermore, a positive correlation between sulfide and [THg] has been found in estuarine and marine pore water (e.g., Bloom et al. 1999).

Levels of MeHg and THg in the epilimnetic and hypolimnetic surface sediment

It may seem surprising that sediment MeHg levels ($[MeHg]_{SE}$) were consistently higher at the shallow station than at the deep station in the upper lake, while the situation was the opposite in the lower lake (Figs. 5*a* and 5*b*). Several independent observations suggested that free sulfide had a significant influence on $[MeHg]_{SE:\ 0\text{–}1\ cm}$ and was one of the variables explaining the fact that MeHg was differently distributed in the two lakes.

At the shallow station in the upper lake, where dissolved oxygen was always >8 ppm in the water above the sediment surface, $[MeHg]_{SE:\ 0\text{–}1\ cm}$ peaked in August, whereas at the deep station where sulfide was present in the bottom water, $[MeHg]_{SE:\ 0\text{–}1\ cm}$ showed a steady decline during July–August (Fig. 5*a*). This decline could be explained by release of MeHg to the water, in line with the fact that [MeHg] increased in the overlying water (Fig. 3*a*).

As stated, release of MeHg from sediment can be a result of depletion of MeHg-scavenging HFO and HMO. The presence of free sulfide indicated that sulfide-reactive HFO and HMO had indeed become depleted in the sediment at the deep station in the upper lake (Fig. 3*c*). It is also possible that the presence of free sulfide had a direct influence on the water solubility of MeHg through the formation of MeHgSH. Using thermodynamic data, Davies-Colley et al. (1985) concluded that metals that form soluble sulfide complexes could be released from anoxic sediment when sulfide-reactive Fe is depleted and free sulfide becomes available for complexation. If this interaction between sulfide-reactive Fe and sulfide affects MeHg in a similar manner, HFO might control MeHg release not only by its ability to bind MeHg but also by its ability to bind sulfide, thereby counteracting the formation of MeHgSH (or any other MeHg sulfide/polysulfide species). Note that the decrease in $[MeHg]_{SE:\ 0\text{–}1\ cm}$ and $[\text{total S}]_{SE:\ 0\text{–}1\ cm}$ coincided and that both [MeHg] and [sulfide] increased in the overlying water when this occurred (Figs. 3*a* and 3*c*). Given that IHg forms complexes with sulfide, its solubility would also be affected by the ability of Fe to scavenge free sulfide.

The decline in $[MeHg]_{SE:\ 0\text{–}1\ cm}$ at the deep station may also have been the result of demethylation. However, given the rapid increase in [MeHg] in the water 0.5 m above the sediment, it seems unlikely that demethylation should be the sole cause (Fig. 3*a*). A net loss of MeHg will result when its rate of formation is lower than its rate of decomposition. When $[HS^-]$ increases, the production of MeHg decreases or even stops (Berman and Bartha 1986; Benoit et al. 1999).

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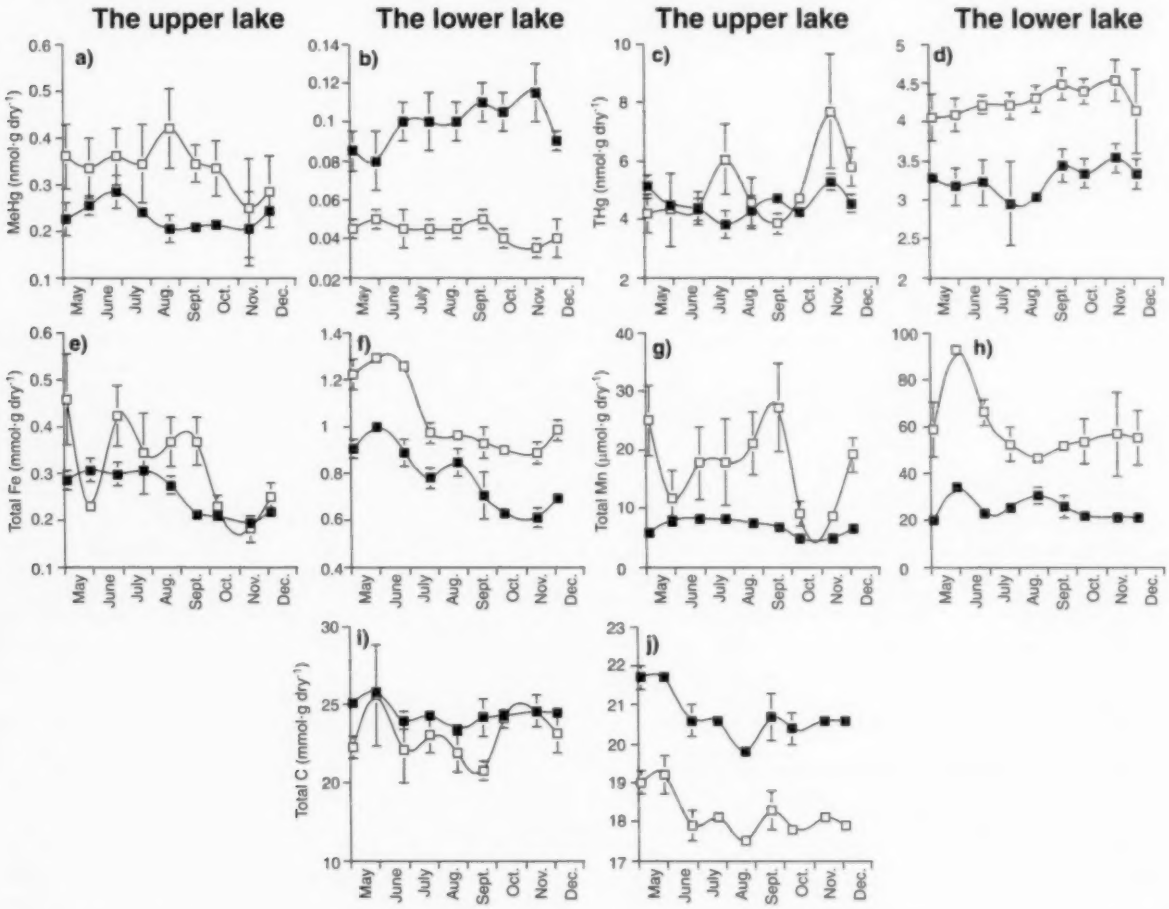
Fig. 5. Temporal variation in [MeHg], [HgT], [total Fe], [total Mn], and [total C] in the surface sediment (0–1 cm) at the deep station (■) and shallow station (□) during 1996 in the (*a*, *c*, *e*, *g*, and *i*) upper lake and (*b*, *d*, *f*, *h*, and *j*) lower lake. Bars represent ±1 SD (n = 4 or 5).



Thus, at high sulfide levels, even a slow rate of decomposition could result in declining $[MeHg]_{SE}$. Hintelmann et al. (2000) showed by using stable Hg isotopes that ambient MeHg in anoxic sediment slurries had a half-life of only 1.8 days. However, they found that sulfide additions totally inhibited MeHg loss. This seemed to contradict the earlier finding that addition of sulfide can convert MeHg to dimethyl mercury (DMeHg) (Wallschläger et al. 1995). According to Craig and Moreton (1984), the reaction sequence for this conversion is $2MeHg^+ + S^{2-} = (MeHg)_2S = HgS + DMeHg$, where the last step occurs upon exposure to light. Thus, the stability of MeHg in sulfide-rich sediment remains uncertain.

The decrease in $[MeHg]_{SE:\ 0\text{–}1\ cm}$ at the shallow station in the fall could have followed changes in the microbial activity, resulting in demethylation exceeding methylation (Fig. 5*a*). Bodaly et al. (1993) found that the methylation to demethylation ratio in lake sediment decreased with decreasing temperature. Possibly, low temperatures are less disfavorable to demethylating organisms than to methylating ones. It is also possible that demethylation is partly an abiotic process (Suda et al. 1991) and hence that it is less depressed at low temperatures than methylation. In the lower lake at the deep station, there was no decline in $[MeHg]_{SE:\ 0\text{–}1\ cm}$ during summer stratification (Fig. 5*b*). Instead, $[MeHg]_{SE:\ 0\text{–}1\ cm}$ increased throughout this period, probably because invariably low sulfide levels in combination with anoxic conditions at the hypolimnetic sediment–water interface favored Hg methylation and millitated against mobilization of MeHg from the sediment.

At the shallow station, low $[MeHg]_{SE:\ 0\text{–}1\ cm}$ (Fig. 5*b*) probably resulted from low net methylation rates. Cellulose fibers, which have been shown to stimulate MeHg production (Regnell et al. 1996) and which probably stimulated MeHg production in the sediment at the shallow station in the upper lake, were not present in the sediment at this station.

THg showed a distribution opposite to that of MeHg in the lower lake, with consistently higher $[THg]_{SE:\ 0\text{–}1\ cm}$ at the shallow station than at the deep station (Figs. 5*b* and 5*d*). Because of the high affinity of Hg for fine organic-rich particles, one would expect sediment focusing to result in higher

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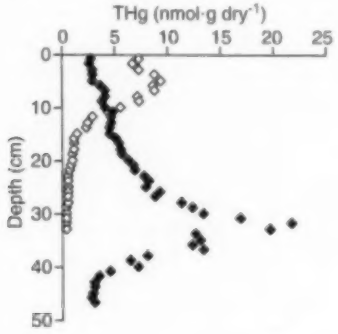
Fig. 6. Stratigraphy of [THg] in the sediment of the lower lake as measured on two sediment cores collected in the fall of 1999. ◆, deep station; ◇, shallow station.



$[THg]_{SE:\ 0\text{–}1\ cm}$ in hypolimnetic than in epilimnetic sediment. Notably, $[total\ C]_{SE:\ 0\text{–}1\ cm}$ was higher at the deep station than at the shallow station, indicating that organic matter was indeed subjected to focusing (Fig. 5*j*). The difference in $[total\ C]_{SE:\ 0\text{–}1\ cm}$ did not seem to be an effect mainly of different organic matter degradation because $[total\ C]_{SE:\ 0\text{–}1\ cm}$ decreased at similar rates at the two stations during summer (Fig. 5*j*).

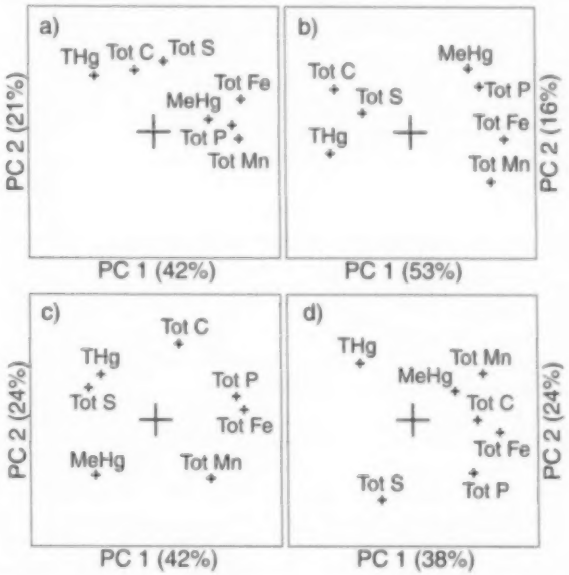
It is evident from the $[THg]_{SE}$ depth profiles in the sediment cores sampled in the fall of 1999 that the major Hg pollution that occurred between 1940 and 1965 still had a strong effect on $[THg]_{SE:\ 0\text{–}1\ cm}$ at the shallow station. At the deep station, the effect of this pollution on $[THg]_{SE:\ 0\text{–}1\ cm}$ was much less because of a much higher sediment burial rate (Fig. 6). Thus, for lakes in which Hg pollution has stopped, sediment focusing could lead to higher [THg] in surface sediment at shallow sites than at deep sites. Of course, bioturbation rates may also affect the rate of burial of old pollution.

Looking at the temporal variation during summer stratification, it is evident that $[THg]_{SE:\ 0\text{–}1\ cm}$ kept increasing at the shallow station while decreasing at the deep station (Fig. 5*d*). This indicated that retention and (or) incorporation of THg was higher at the shallow station, in line with the fact that Hg seemed to be released from particulate matter in conjunction with anaerobic conditions (Fig. 3). Thus, a higher retention of Hg could have contributed to the fact that $[THg]_{SE:\ 0\text{–}1\ cm}$ was higher at the shallow station than at the deep station.

Relationships between THg, MeHg, and other constituents in the sediment

Correlation analysis did not reveal any statistically significant ($P < 0.05$) temporal covariation between either THg or MeHg and the other sediment variables at any of the four stations, but the statistical power was low because of the high number of comparisons tested in each matrix ($n = 11$) and because sampling was restricted to nine occasions. We do not present these data because the pairwise relationships between the sediment variables were well reflected by the PCA plots (Fig. 7). In the PCAs, individual values instead of mean values were used. Thus, small-scale spatial variation was included in the calculation. However, the PCA plots were only slightly altered using mean values instead of individual ones (data not shown).

Fig. 7. Plots based on PC models, the variables being those indicated in the figure and the objects being the sediment samples (0–1 cm) ($n = 41$ or 42) taken at the (*a*) upper lake deep station, (*b*) upper lake shallow station, (*c*) lower lake deep station, and (*d*) lower lake shallow station. The samples were taken on nine different occasions (four or five replicate samples on each occasion) at approximately monthly intervals during 1996. All variables were standarized (SD = 1) prior to performing the PCA. The variance explained by each PC, expressed as a percentage of total variance, is given in parentheses. The large cross indicates the origin.



The PCA suggested that THg (essentially IHg) was bound to organic matter in the upper lake (Figs. 7*a* and 7*b*), indicating that most of the THg was still associated with cellulose fibers and that the content of fiber explained most of the variation in $[THg]_{SE:\ 0\text{–}1\ cm}$. Possibly, lignin residues, degradation products, and associated microorganisms increased the Hg-binding capacity of the fibers (Ziomek and Williams 1989). It is also possible that Hg was present as inorganic sulfide. The projection of total S was closer to that of total C than that of total Fe (Figs. 7*a* and 7*b*), suggesting that most of the S content was organically bound and hence that Hg was bound to organic matter via reduced S ligands. However, a positive relationship between organic matter and total S could also result from a stimulation of sulfate reduction and FeS(s) formation by organic matter.

MeHg seemed to be associated mainly with Fe and Mn, not with organic matter or sulfide, at both stations in the upper lake (Figs. 7*a* and 7*b*). This indicated that once methylated, Hg had higher affinity for redox-sensitive HFO and HMO than for other sediment constituents, in accordance with the fact that [MeHg] was strongly correlated with [total Fe] and [total Mn] in the anoxic hypolimnetic water (Table 3). Apparently, methylation of Hg, which in this case probably was coupled to anaerobic degradation of the fibers (Regnell et al. 1996), resulted in a transfer of Hg to HFO

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and HMO. One would expect that IHg was released as a result of organic matter decomposition, forming either DOC or sulfide complexes, and that IHg was also scavenged by HFO and HMO. Possibly, the fraction of the sediment THg that was released was too small to result in a positive correlation between $[THg]_{SE:\ 0\text{–}1\ cm}$ and the variables $[\text{total Fe}]_{SE:\ 0\text{–}1\ cm}$ and total $[Mn]_{SE:\ 0\text{–}1\ cm}$. Gobeil and Cossa (1993) found that Hg mobilized through diagenetic processes was negligible compared with the total content of Hg in Hg-polluted estuarine sediments in the Laurentian Trough, Canada.

At both stations in the upper lake, $[\text{total P}]_{SE:\ 0\text{–}1\ cm}$ was the sediment variable showing the strongest covariation with $[MeHg]_{SE:\ 0\text{–}1\ cm}$ (Figs. 7*a* and 7*b*). In addition to MeHg and P being associated with Fe, the level of both may have decreased because of increasing sulfide levels. Phosphorus is known to be displaced from Fe when the latter is converted to FeS (Roden and Edmonds 1997). MeHg may not be displaced to the same extent as a result of this conversion. Krabbenhoft et al. (1998) presented results indicating that FeS(s) has the ability to scavenge MeHg. However, when all sulfide-reactive Fe is depleted, free sulfide may compete with FeS(s) in the binding of MeHg. Furthermore, free sulfide inhibits MeHg production. Thus, released MeHg will not be readily restored by new production. Another explanation for a positive P–MeHg relationship is that P may stimulate activities of methylating bacteria.

In the lower lake, the absence of Hg-polluted cellulose fibers may explain the weak relationship between $[THg]_{SE:\ 0\text{–}1\ cm}$ and $[\text{total C}]_{SE:\ 0\text{–}1\ cm}$ (Figs. 7*c* and 7*d*). However, because of the high affinity of Hg for organic matter, one would have expected $[THg]_{SE:\ 0\text{–}1\ cm}$ and $[\text{total C}]_{SE:\ 0\text{–}1\ cm}$ to covary irrespective of the presence of cellulose fibers. A process that may counteract a tendency for a positive relationship between these variables is degradation of organic matter because this process will lead to a higher $[THg]_{SE:\ 0\text{–}1\ cm}$ and to a lower $[\text{total C}]_{SE:\ 0\text{–}1\ cm}$, provided that Hg is retained in higher proportion than the organic matter. At the shallow station, it was evident that $[\text{total C}]_{SE:\ 0\text{–}1\ cm}$ decreased during summer, presumably because of organic matter degradation, whereas $[THg]_{SE:\ 0\text{–}1\ cm}$ increased. At the deep station, organic matter degradation in the surface sediment seemed to have occurred at a rate similar to that at the shallow station, but $[THg]_{SE:\ 0\text{–}1\ cm}$ decreased during summer, indicating that Hg was released (Figs. 5*d* and 5*j*).

Apparently at odds with HFO and HMO being important scavengers of dissolved Hg(II), $[\text{total Fe}]_{SE:\ 0\text{–}1\ cm}$ and $[\text{total Mn}]_{SE:\ 0\text{–}1\ cm}$ were weakly or even inversely related to $[THg]_{SE:\ 0\text{–}1\ cm}$ at both stations in the lower lake (Figs. 7*c* and 7*d*). At the shallow station, it is likely that the amount of HFO and HMO in the surface sediment was not limiting for the retention and (or) incorporation of Hg, i.e., that there was always enough oxide to efficiently retain it. Thus, one should hardly expect $[\text{total Fe}]_{SE:\ 0\text{–}1\ cm}$ and $[\text{total Mn}]_{SE:\ 0\text{–}1\ cm}$ to show strong relationships with $[THg]_{SE:\ 0\text{–}1\ cm}$ at this station. Furthermore, most of the THg was probably bound to particulate organic matter, from which only a small fraction was transferred to HFO and HMO (Gobeil and Cossa 1993). At the deep station, THg may have been in the form of sulfide (Fig. 7*c*).

$[MeHg]_{SE:\ 0\text{–}1\ cm}$ showed a relationship with $[\text{total Fe}]_{SE:\ 0\text{–}1\ cm}$ and $[\text{total P}]_{SE:\ 0\text{–}1\ cm}$ at the deep station in the lower lake that was totally different from that seen in the upper lake. Instead of clustering with total Fe and total P in the PCA, the projection of MeHg was opposing those of total Fe and total P, suggesting that $[MeHg]_{SE:\ 0\text{–}1\ cm}$ varied inversely with $[\text{total Fe}]_{SE:\ 0\text{–}1\ cm}$ and $[\text{total P}]_{SE:\ 0\text{–}1\ cm}$ (Fig. 7*c*). This indicated that the retention of MeHg and inhibition of methylation by sulfide were not limiting factors for $[MeHg]_{SE:\ 0\text{–}1\ cm}$, consistent with the fact that sulfide could not be detected in the hypolimnion. Under these conditions, i.e., when there is a surplus of sulfide-reactive Fe over sulfide, inhibition of Hg methylation and complexation of MeHg by sulfide would be low. The inverse relationship between $[\text{total Fe}]_{SE:\ 0\text{–}1\ cm}$ and $[MeHg]_{SE:\ 0\text{–}1\ cm}$ could be a result of reductive release of Fe and methylation of Hg(II) both occurring under anoxic conditions (Figs. 5*b* and 5*f*). The negative correlation between MeHg and total P is consistent with the fact that P is released when HFO is converted to FeS(s) by sulfate-reducing bacteria, known also to methylate Hg(II). A likely explanation for the fact that the two study lakes displayed different MeHg–P–Fe relationships was that sulfide-reactive Fe was depleted in the upper lake but not in the lower lake.

THg in the surface sediment and the settling matter

In both lakes, $[THg]_{SE:\ 0\text{–}1\ cm}$ was higher than the integrated [THg] ($[THg]_{INT}$) in the settling matter trapped 1 m above the sediment surface, except at the deep station in the upper lake (Fig. 8). At the latter station, $[THg]_{SE:\ 0\text{–}1\ cm}$ and $[THg]_{INT}$ were similar, probably because of high resuspension, especially in conjunction with turnover. For the deep station in the lower lake, with a well-buried Hg peak and with only slightly increasing [THg] with increasing sediment depth in the upper 0- to 10-cm layer (Fig. 6), it is questionable whether "old Hg" could explain the fact that $[THg]_{SE:\ 0\text{–}1\ cm}$ was 1.5 times higher than $[THg]_{INT}$ at the end of the study period. An alternative explanation is postdepositional enrichment, i.e., direct sorption of dissolved or colloidal Hg by the sediment surface. The hypolimnetic Hg enrichment (Fig. 9) indicated that the settling matter became depleted in Hg when it reached the anoxic hypolimnion. Upon reoxygenation, it seems possible that these particles would be undersaturated with Hg in relation to dissolved or colloidal Hg and that this would lead to direct sorption. Any such Hg could be released during the next anaerobic event, but perhaps not all of it.

In principle, direct sorption could have occurred at the other stations as well, depending on how fast equilibrium partitioning is reached, the presence of dissolved Hg, the time it takes for particles to reach the sediment, and how stable are those conditions that affect the partitioning of Hg between particulate and dissolved forms.

Whole-lake studies performed in the Experimental Lakes Area, Canada, did suggest that for heavy metals reaching the epilimnion in a dissolved state, direct sorption could be significant (e.g., Hesslein 1987). Watras et al. (1995*b*) found that on average, 80% of THg passed 0.45-μm filters in epilimnetic water from 23 lakes in Wisconsin. As already noted, organic matter decomposition would result in higher $[THg]_{SE}$, provided that Hg is more conservative than the organic matter during sediment diagenesis. However, only at the shallow station in the lower lake was there a significant

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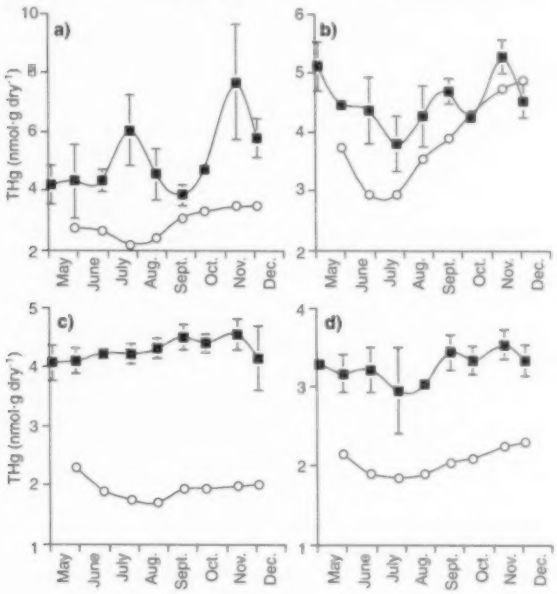
Fig. 8. Temporal variation in [THg] in the surface sediment (0–1 cm) (■) and in the integrated [THg] (cumulative amount of HgT deposited/cumulative amount of dry substance deposited) in the settling matter (○) collected 1 m above the sediment–water interface during 1996 at the (*a*) upper lake shallow station, (*b*) upper lake deep station, (*c*) lower lake shallow station, and (*d*) lower lake deep station. Bars represent ±1 SD (n = 4 or 5). The values for settling matter are based on single samples.



difference between [total C]$_{SE}$ and [total C]$_{INT}$ (data not shown).

Effects of the sediment on the hypolimnetic Hg

The water depth profiles suggest that release from sediment was more important for MeHg than for IHg, especially in the upper lake (Fig. 10). However, as already mentioned, an apparent slow release of IHg from the sediment and from settling particles in the upper lake could be a result of rapid methylation.

In the upper lake, the decrease in [MeHg]$_{SE: 0–1 cm}$ could at most explain 60% of the major increase in MeHg in the 1-m water layer closest to the sediment, provided that new production of MeHg in the sediment was negligible (Fig. 3*a*), leaving most of the buildup of MeHg in the anoxic bottom water unexplained. The contribution from the sediment could have been even less if the decrease in [MeHg]$_{SE: 0–1 cm}$ was the result of demethylation rather than of release of MeHg to the water. On the other hand, sediment at depths greater than the sampled layer could be an additional source of MeHg. In the lower lake, [MeHg]$_{SE: 0–1 cm}$ increased rather than decreased during the buildup of MeHg in the hypolimnion (Fig. 3*g*).

High-resolution sampling close to the sediment–water interface would have been necessary to determine the flow of MeHg and IHg between sediment and water. Whether or not MeHg produced in the sediment constituted a significant part of the MeHg present in the anoxic bottom water, sedi-

Fig. 9. Temporal variation in [MeHg] and [IHg] in epilimnetic and hypolimnetic water (single samples) during 1996 in the (*a* and *b*) upper lake and (*c* and *d*) lower lake. □, 0.5 m for both lakes; ■, 6.5 and 11.5 m for the upper lake and lower lake, respectively.

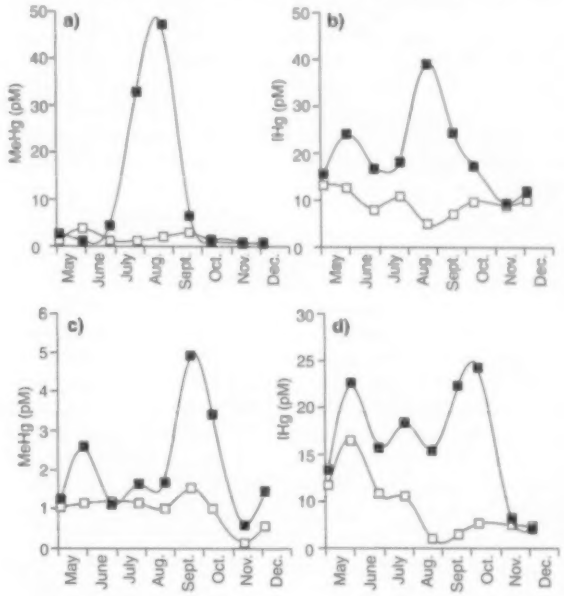
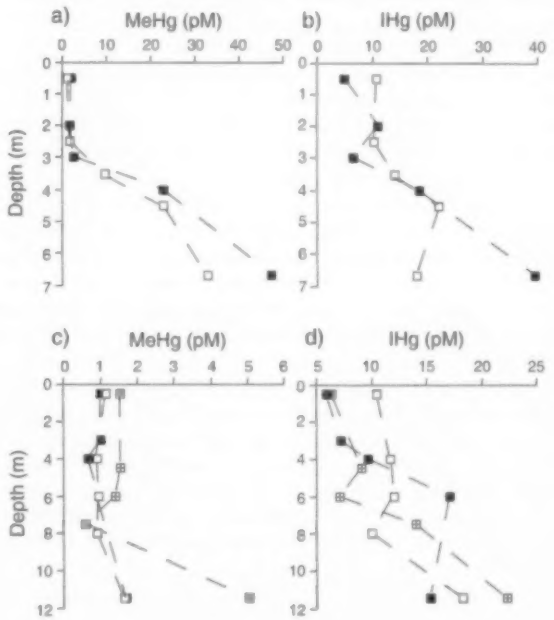


Fig. 10. Depth profiles of [MeHg] and [IHg] in the water column of the (*a* and *b*) upper lake and (*c* and *d*) lower lake on the dates indicated below (1996). The values are based on single samples. □, July 24; ■, August 19; ⊞, September 17.



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ment oxygen consumption and release of nutrients may have contributed significantly to the conditions in the overlying water that promoted both Hg methylation and release of MeHg from settling particles.

In the upper lake, [MeHg] reached >50% of [THg] in the hypolimnetic water and $[MeHg]_{SE}$ reached >10% of $[THg]_{SE}$, whereas in the lower lake, the corresponding percentages were 18 and 3%. This indicated that the presence of cellulose fibers in the sediment greatly stimulated MeHg production in the upper lake, in agreement with a laboratory study of sediment and water from another lake (Regnell et al. 1996).

Conclusions

The presence of MeHg in the water was more redox sensitive than that of IHg, as evidenced by the fact that [total Fe] showed stronger correlation with [MeHg] than with [IHg] in the hypolimnetic water of both lakes. There were two possible links between [MeHg] and [total Fe] in the hypolimnetic water: (*i*) MeHg and its complexing ligands were sorbed by HFO; when HFO was reductively dissolved, leading to an increase in [total Fe], the solubility of MeHg increased and (*ii*) MeHg production was caused by the same microbial activity that caused dissolution of HFO, or coincided with it.

In the sediment of the upper lake, total Fe and Mn were positively correlated with MeHg because (*i*) MeHg was bound to HFO, HMO, and FeS(s), (*ii*) HFO and HMO made Hg more available for methylation by lowering free sulfide levels, and (*iii*) by scavenging free sulfide and dissolved organic matter, HFO and HMO decreased the MeHg-complexing capacity of the water in contact with the sediment.

In the lower lake, there was an inverse relationship between MeHg and total Fe in the sediment at the deep station because anaerobic microbial activity caused reductive dissolution of HFO (leading to decreased levels of total Fe in the sediment) as well as increased net MeHg production (leading to increased levels of MeHg in the sediment). The effects of Fe described in points *i–iii* in the previous paragraph were never limiting for the retention or production of MeHg in the lower lake because of a constant surplus of sulfide-reactive Fe in relation to sulfide.

HFO and HMO were important scavengers of IHg also, as evidenced by the fact that [IHg], [total Fe], and [total Mn] increased simultaneously in the hypolimnion of the lower lake. The fact that [IHg] increased later than [MeHg], [total Fe], and [total Mn] in the hypolimnion of the the upper lake was a result of initial depletion of mobilized IHg by methylation.

We believe that our findings are valid for lakes that have received organic wastes or are naturally eutrophic in which anoxic conditions develop at the sediment–water interface. Especially in riverine lakes, factors controlling net sedimentation and net methylation of Hg are of high environmental concern because efficient withdrawal of IHg and MeHg in such lakes would lessen the Hg exposure of the biota downstream.

Our interpretation of the present results is, of course, not necessarily correct. To gain further information, we would like to go on with studies of phase associations of Hg in the sediment and in the water column and with studies of demethylation of MeHg.

Acknowledgments

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Recent declines in benthic macroinvertebrate densities in Lake Ontario[1]

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Abstract: Surveys of benthic macroinvertebrates conducted in Lake Ontario during 1994 and 1997 revealed recent declines in populations of three major taxonomic groups: Oligochaeta, Sphaeriidae, and *Diporeia* spp. (Amphipoda), with the most drastic reductions occurring in the latter. Results from sediment measurements were used to classify deepwater sediments into three habitat zones. Densities of all three taxa declined in the shallowest (12–88 m) of the sediment zones between 1994 and 1997; the greatest changes in density were observed for *Diporeia*, which declined from 3011 to 145 individuals·m^{-2}, and for total benthic macroinvertebrates, which declined from 5831 to 1376 individuals·m^{-2}. Mean densities of *Dreissena* spp. in 1997 were highest in the shallowest zone, and the areas of greatest densities corresponded to areas of largest reductions in *Diporeia* populations. We believe that dreissenids are competing with *Diporeia* by intercepting the supply of fresh algae essential for *Diporeia* survival. A decline in macroinvertebrate densities, especially populations of an important food item such as *Diporeia*, in Lake Ontario sediments at depths of 12–88 m may have a detrimental impact on the benthic food web.

Résumé : Des inventaires des macroinvertébrés benthiques du lac Ontario en 1994 et 1997 ont révélé des déclins récents dans les peuplements de trois importants groupes taxonomiques, les oligochètes, les sphaeriidés et, de façon encore plus marquée, les amphipodes *Diporeia* spp. Des analyses de sédiments ont permis de classifier les substrats de la zone profonde en trois types d'habitats. Les densités des trois taxons ont diminué dans la zone la moins profonde (12–88 m) de 1994 à 1997; les déclins les plus importants se sont manifestés chez les *Diporeia*, don't les densités sont passées de 3011 à 145 individus·m^{-2}, et dans le peuplement total de macroinvertébrés qui a baissé de 5831 à 1376 individus·m^{-2}. Les densités moyennes les plus élevées de *Dreissena* spp. en 1997 ont été retrouvées dans la zone la moins profonde, leurs densités maximales correspondant aux régions des plus forts déclins des peuplements de *Diporeia*. Nous croyons que les dreissénidés font compétition aux *Diporeia* en leur bloquant l'accès aux algues fraîches nécessaires à leur survie. Un déclin des densités des macroinvertébrés, particulièrement celui d'une ressource alimentaire importante comme les peuplements de *Diporeia*, dans les sédiments du lac Ontario aux profondeurs 12–88 m peut avoir un effet néfaste sur le réseau alimentaire benthique.

[Traduit par la Rédaction]

Introduction

The deepwater, benthic macroinvertebrate community of the Great Lakes is dominated by a few species of organisms. The cold–stenotherm association includes Oligochaeta, Sphaeriidae, and *Diporeia* spp. (Cook and Johnson 1974). Two species of oligochaetes, *Stylodrilus heringianus* and *Limnodrilus hoffmeisteri*, are found throughout the offshore regions and may account for 10–20% of the total benthic population density (Mozley and Howmiller 1977). Members of the bivalve family Sphaeriidae are usually found in waters less than 90 m deep and account for 5–15% of the total benthic population density. The amphipod *Diporeia* spp. (formerly *Pontoporeia hoyi*) is the most abundant macroinvertebrate (Mozley and Howmiller 1977; Nalepa 1991) in the Great Lakes. In deeper water habitats, it accounts for 40–70% of the total density of benthic organisms (Nalepa 1991), reaching greatest densities at depths below the summer thermocline in waters 30–60 m deep.

In the Great Lakes, *Diporeia* is found in greatest numbers in soft or fine sediments and in sand overlain with a thin layer of detritus (Marzolf 1965). An important source of food for *Diporeia* arrives in the spring and fall when water conditions favor diatom blooms and subsequent settling of diatoms onto the sediments (Saether 1980). *Diporeia* depends on settling algae for growth and reproduction, storing

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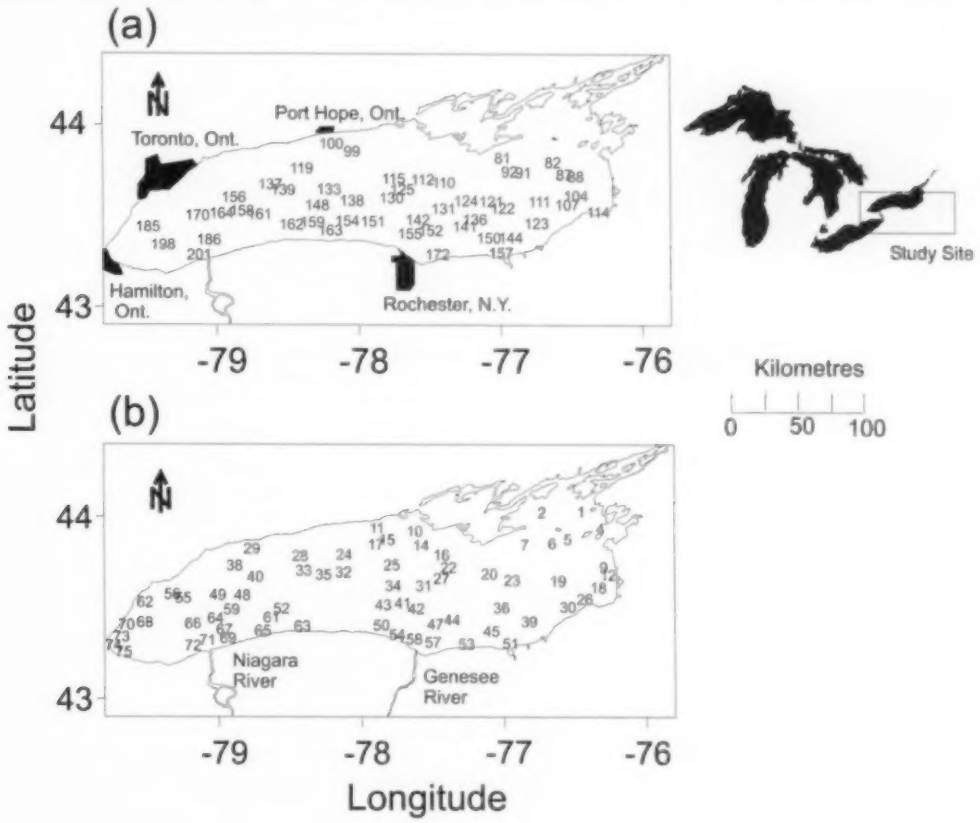
Fig. 1. Location of sampling stations in Lake Ontario in (*a*) 1994 and (*b*) 1997.



the assimilated energy as lipid. Lipid levels of *Diporeia* are high compared with those of other Great Lakes benthic macroinvertebrates and can account for up to 50% of the amphipods' dry weight after the spring diatom bloom (Gardner et al. 1985). It has been estimated that within deeper water habitats of the Great Lakes, 23% of the total annual carbon production from phytoplankton is consumed by this organism (Flint 1986). Fitzgerald and Gardner (1993) reported that *Diporeia* ingested 61% of the spring diatom bloom in Lake Michigan. A large portion of *Diporeia* biomass is transferred up the food chain to forage fish. *Diporeia* is the dominant food organism of the slimy sculpin in Lake Ontario (Owens and Weber 1995) and most other fish species feed on *Diporeia* at some stage in their life cycle (Mozley and Howmiller 1977). Because of the importance of benthic macroinvertebrates and particularly *Diporeia* in fish diets, changes in the status of *Diporeia* or the other two major taxa could have dramatic effects on fish production in the Great Lakes.

Colonization by the dreissenid mussels *Dreissena polymorpha* and *Dreissena bugensis* is probably one of the more ecologically important events to occur in the Great Lakes during the last decade (MacIsaac 1996). Since their discovery in 1988, dreissenids have colonized both soft and hard substrates to depths of 130 m (Mills et al. 1993) and reached densities of 340 000·m^{-2} in some areas (Leach 1993). Because of high densities, widespread distribution, and high filtering rates, *Dreissena* populations can filter large volumes of water and have the capacity to decrease algal biomass (Holland 1993; Fahnenstiel et al. 1995). Therefore, this diversion of algal production into dreissenid tissue and biodeposits may deprive *Diporeia* and other deepwater macroinvertebrates of food settling from the water column.

In this study, we examined changes in densities of *Diporeia*, Oligochaeta, and Sphaeriidae in Lake Ontario between 1994 and 1997 and relate these changes to those observed over the past three decades. We present evidence that all three taxonomic groups have declined in density in waters between 12 and 88 m at a time when *Dreissena* densities were increasing.

Methods

Macroinvertebrate sampling design and processing

Benthic samples were collected from Lake Ontario in late August 1994 and early September 1997 at 55 and 75 stations, respectively (Fig. 1). Sampling depths ranged from 12 to 213 m. Station locations in 1994 and 1997 were based on a randomized design using a systematic grid (Stevens 1997). Locations in 1994 were then classified into habitat zones based on principal components analysis (PCA) of physical and chemical data (see below). Because only a subset of physical and chemical measurements were taken in 1997,

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Table 1. Physical and chemical characteristics of Lake Ontario sediments from the 1994 survey by three sediment classifications; percentage variation and loadings for the first two axes of the principal components analysis (PCA) for physical and chemical characteristics.

		Physical parameters					Chemical parameters			Survival (%)	
Sediment zones	No.	Depth (m)	Sand (%)	Silt (%)	Clay (%)	Carbon (%)	NH_3 ($\mu g \cdot L^{-1}$)	Sulfide ($\mu g \cdot L^{-1}$)		*Chironomus*	*Hyallela*
Low-depositional	10	45	74.7 (5.32)	23.8 (4.94)	0.21 (0.04)	1.65 (0.43)	4.28 (1.88)	1.12 (1.10)		93.5 (2.84)	97.6 (0.69)
Transitional	7	81	29.9 (4.41)	67.4 (4.30)	0.43 (.12)	3.23 (0.38)	1.78 (0.30)	14.3 (11.4)		97.5 (0.77)	96.8 (1.41)
High-depositional	34	147	13.2 (2.12)	82.4 (2.11)	1.03 (0.07)	3.24 (0.17)	1.09 (0.21)	2.13 (1.48)		93.1 (1.33)	93.2 (1.07)
	Principal component loadings								Survival (%)		
PCA		Depth (m)	Sand (%)	Silt (%)	Clay (%)	Carbon (%)	NH_3 ($\mu g \cdot L^{-1}$)	Sulfide ($\mu g \cdot L^{-1}$)		*Chironomus*	*Hyallela*
1st Axis (40)[a]		0.83	–0.96	0.96	0.78	0.65	–0.51	0.04		–0.45	–0.06
2nd Axis (14)[a]		–0.96	–0.15	0.15	–0.08	0.43	0.41	0.57		0.36	0.72

Note: No. = number of stations located in each sediment zone. Depth and physical and chemical values are means (±SE).
[a]Values in parentheses represent percentage of variation explained.

classification of these locations was based on grain size and depth, two of the most important discriminating measurements as determined by PCA. Position for sampling was determined by a Loran C navigation.

At each station, three samples were taken with a Ponar grab (area = 0.048 m^2); the samples were pooled into a single sample, placed into an elutriation device, and washed through a nylon sleeve with a 500-µm opening (Mozley and Howmiller 1977). The residue retained was preserved in 5% formalin containing rose bengal stain. In the laboratory, organisms were initially removed from samples under a dissecting microscope and identified into major taxonomic groups. Later, organisms were identified to the lowest possible taxonomic level. *Dreissena* were counted and identified to species in 1997 but not in 1994.

Sediment collection and analysis

In 1994, an additional Ponar grab sample was collected from each station and sediment from the top 2 cm, assumed to represent the biologically active zone, was placed into clean glass containers and kept at 5°C. These sediments were analyzed for grain size, organic carbon, ammonia, sulfide, and relative toxicity. Standard methods for grain size analysis and total organic carbon were followed (ASTM 1991). Results for grain size were reported as percentage clay (<4 µm), silt (4–60 µm), and sand (>60 µm). Ten-day toxicity tests for *Hyalella azteca* and *Chironomus tentans* were conducted according to standard methods (Ingersoll and Nelson 1990). Results were reported as percentage survival of organisms. Measurements of pore-water ammonia and sulfide concentrations followed methods of Ankley et al. (1993).

Statistical analysis

A principal components analysis (PCA, Wilkinson 1996) was performed using physical and chemical characteristics of sediments collected in 1994 to identify different macroinvertebrate habitat zones. Variables included in the analysis were percentage survival of *Hyallela azteca* and *Chironomus tentans* in toxicity tests, percentage sand, silt, clay, and carbon, depth, ammonia, and sulfide. Mean population densities in each zone (numbers of individuals·m^{-2}) and between year(s) were compared with analysis of variance (ANOVA) and Tukey's test ($p \leq 0.05$) using densities at each station as replicates (Wilkinson 1996). Water depth was included as a covariate term. Variance stabilizing transformations were used prior to data analysis. An arcsine – square root transformation was used for percentage data and a log transformation was used for macroinvertebrate density data.

Results

Sediment analysis

Results from PCA indicate that the first principal component accounted for 40% of total variability with depth, % sand, and % silt being the variables that correlated most highly with the first axis (Table 1). In general, sand and silt accounted for the majority of dry sediment mass at all stations, with clay comprising <2% of the remaining sediment mass at each site. Thus deepwater stations with high amounts of silt and clay are located to the right of where the PC1 and PC2 axes intersect (Fig. 2). As a result, a strong negative correlation exists between sand and silt such that areas with high % sand have low % silt, and vice versa. The second principal component accounted for 14% of the variability with percentage survival of *H. azteca*, ammonia, and sulfide levels being most highly correlated with the second axis.

The results of PCA for habitat characteristics with the 1994 data revealed three groupings of stations (Table 1, Fig. 2).

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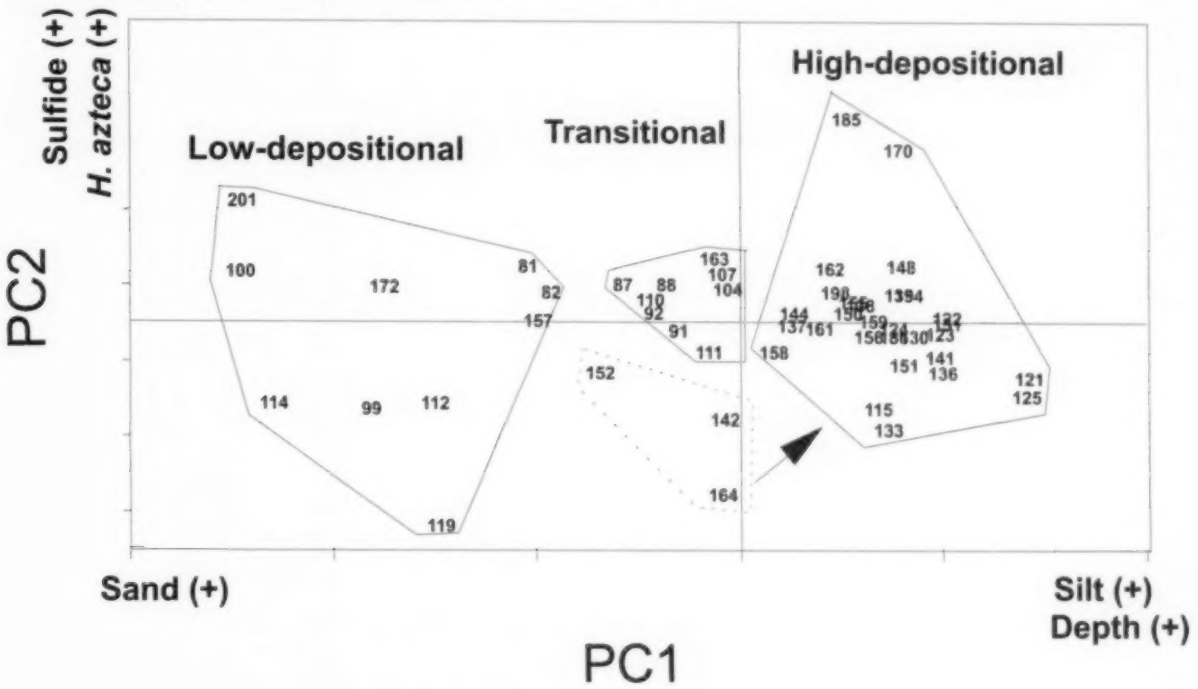
Fig. 2. Classification of Lake Ontario sediments by principal components analysis (PCA) for physical and chemical parameters, 1994. Variables used in PCA included depth, NH_3, and sulfide ($\mu g \cdot L^{-1}$), survival of *Hyalella azteca* and *Chironomus tentans* (percentage survival), and percentage sand, silt, clay, and carbon.



The three groupings were identified as low-depositional, transitional, and high-depositional areas (Table 1, Figs. 1, 2). The low-depositional zone included stations with a high ratio of sand to silt and low % carbon; these stations were generally located in shallower, nearshore regions (12–88 m). The high-depositional zone was characterized by stations with sediments of a higher ratio of silt to sand and high % clay relative to sediments from other stations. High-depositional stations were deeper (90–213 m) and located centrally in the lake. The transitional zone included stations with sediments having a mixture of sand and silt and % clay that was intermediate between low- and high-depositional stations. Depths in this zone were intermediate (63–91 m) and stations were located between the low-depositional and high-depositional zone (Fig. 1, 2). Three stations (stations 142, 152, and 164) were intermediate in sand and silt but located in deeper water, i.e., greater than 134 m (Fig. 1). These stations were located on the Whitby–Olcott and Scotch Bonnet sills (Thomas et al. 1972) that separate the Lake Ontario basin into three deepwater high-depositional zones. For macroinvertebrate analysis, stations located on the sills were placed in the high-depositional group.

Macroinvertebrate densities

Three taxa, *Diporeia* spp., Oligochaeta, and Sphaeriidae, comprised 91–99% of all macroinvertebrates collected in 1994 and 1997. There were significant differences for major taxa within sediment zones. In the low-depositional zone, densities of *Diporeia* were most abundant at depths greater than 42 m in 1994 but no pattern was discernable along the depth gradient sampled in 1997 (Table 2). At stations where *Diporeia* were abundant, densities averaged $6363 \cdot m^2$ in 1994 compared with $954 \cdot m^2$ in 1997. Further, the percentage of stations where no or very few *Diporeia* (<5 m^2) were found increased from 40% in 1994 to 84% in 1997. In the transitional and high-depositional zones, there was little association between depth and *Diporeia* densities in 1994 or 1997, and at stations where *Diporeia* were abundant, densities were comparable. There were no apparent patterns between densities of Oligochaeta or Sphaeriidae and depth within any of the three sediment zones.

Results from ANOVA of mean densities with main effects of differences between years and sediment zones were statistically significant for all three taxa and total macroinvertebrate density with p-values of 0.001–0.008. The covariate term water depth was never a significant factor in the ANOVA with p-values of 0.146–0.504. Densities of *Diporeia*, Oligochaeta, and Sphaeriidae and total macroinvertebrates were significantly (Tukey's test) lower in 1997 compared with 1994 in the low-depositional zone (Table 3), with percentage declines of 95, 61, 45, and 76%, respectively. Densities of Oligochaeta, but not *Diporeia* and Sphaeriidae, were significantly lower in 1997 compared with 1994 in the high-depositional zone, with a percentage decline of 58%. Another general trend, although not always statistically significant, was for densities to be lower in the high-depositional zone compared with the other two sediment zones for all three taxa and total macroinvertebrates (Table 3). In the transitional zone, there were no significant differences in densities of the three taxa and total abundance

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Table 2. Densities (no.·m^{-2}) of four major taxa in Lake Ontario are ordered by depth and grouped by sediment zones.

		1994					1997			
Depth (m)	Station	*Diporeia*	Oligochaeta	Sphaeriidae	Depth (m)	Station	*Diporeia*	Oligochaeta	Sphaeriidae	*Dreissena*
Low-depositional zone										
12	114	0	530	155	14	1	0	0	0	1511
12	201	185	5671	237	15	15	276	800	516	920
20	172	0	5662	103	18	75	0	4129	467	93
23	157	0	525	22	19	51	0	342	0	2587
42	81	4	3907	947	20	74	0	2071	280	173
48	100	7528	1277	1477	23	63	0	2218	4	2133
59	82	2054	1369	588	25	30	0	187	0	5640
59	99	6473	1036	327	26	2	4	116	0	49
83	112	6983	1036	207	26	29	4	111	316	22
88	119	5952	1603	172	27	4	0	111	0	9
					27	26	0	53	0	258
					27	62	0	369	440	4
					29	58	0	3280	0	3480
					31	10	0	0	0	2022
					31	54	0	2062	4	1987
					33	12	0	173	18	13
					37	11	0	284	58	3964
					38	9	0	133	9	147
					40	53	4	236	378	1089
					40	73	0	893	462	0
					43	6	0	378	84	0
					45	70	1138	2191	2431	18
					46	7	0	409	40	0
					49	5	0	409	0	9
					51	14	453	649	649	111
					56	16	1564	711	449	187
					57	57	0	542	204	36
					58	18	0	796	169	53
					62	17	1058	449	307	311
					64	65	0	849	0	53
					69	69	0	2053	142	756
Transitional zone										
70	163	1322	960	831	63	22	0	520	98	0
75	88	5267	960	771	74	71	3640	2009	200	13
76	87	6202	1069	952	75	72	1729	764	231	0
82	91	7778	501	646	76	28	3751	809	142	9
82	92	7890	681	573	80	38	6280	1307	4	0
89	185	4556	319	134	81	24	5364	849	111	4
96	110	4763	184	138	83	68	3453	227	98	0
					87	56	8813	1324	316	0
					90	50	0	320	93	0
					91	20	436	209	164	0
High-depositional zone										
93	198	2976	440	108	100	67	1760	280	178	0
100	186	5110	511	174	112	25	2636	107	4	4
103	144	4	430	297	112	33	773	129	22	0
114	107	0	1783	332	122	66	1729	196	36	0
115	104	0	610	263	125	64	996	364	40	0
117	159	1848	166	39	130	48	1107	53	40	0
119	137	2407	199	17	134	19	533	178	138	0
122	155	3062	922	168	134	31	2369	142	31	0
125	115	3618	407	34	136	49	778	18	4	0
133	154	2692	345	82	138	23	1062	111	22	0
134	139	426	270	4	138	59	1831	360	27	0

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Table 2 (*concluded*).

		1994					1997			
Depth (m)	Station	*Diporeia*	Oligochaeta	Sphaeriidae	Depth (m)	Station	*Diporeia*	Oligochaeta	Sphaeriidae	*Dreissena*
134	164	2192	199	43	139	35	1387	173	18	0
134	170	2588	851	65	140	32	1582	49	0	0
136	156	1507	175	34	143	45	0	218	111	0
139	158	1658	199	56	147	61	1853	671	89	4
140	151	1848	445	30	155	42	409	204	4	0
141	162	1141	558	56	158	39	0	249	89	0
143	150	1447	1339	146	159	34	1591	204	0	0
145	125	1537	95	9	160	52	693	133	4	0
145	152	1809	899	26	163	43	1569	236	18	0
148	124	2519	142	90	170	41	1436	178	4	0
152	133	1451	180	22	175	47	2036	267	0	0
152	161	1757	317	13	200	44	689	71	9	0
156	142	1537	563	34	209	36	458	89	0	0
160	130	1960	572	13	175	47	2164	283	0	0
165	148	2347	662	22	200	44	732	76	9	0
171	131	1891	468	52	209	36	487	94	0	0
175	121	1826	307	9						
180	138	1899	866	22						
195	122	1624	525	26						
196	141	1856	416	0						
203	111	1662	464	4						
210	136	1873	255	0						
213	123	4	951	185						

Table 3. Comparison between densities (no.·m^{-2}) of three major taxa and total invertebrate densities in Lake Ontario by year and three sediment zones.

	Low-depositional zone		Transitional zone		High-depositional zone	
Taxa	1994	1997	1994	1997	1994	1997
No. of stations	10	31	7	10	34	27
Diporeia	3011 (1098) a	145 (69) b	5570 (876) a	3347 (920) a	1884 (184) a	1419 (190) a
Oligochaeta	2125 (603) a	830 (185) bc	631 (121) abc	874 (182) ab	484 (58) b	203 (27) c
Sphaeriidae	437 (150) a	240 (81) bc	596 (127) a	146 (27) ab	75 (15) bc	35 (8.8) c
Total macroinvertebrate	5831 (1013) a	1376 (282) b	6898 (902) a	4358 (1055) ac	2514 (181) ac	1689 (197) c

Note: Values are means (±SE). Values followed by a different letter are significantly different (ANOVA followed by Tukey's test, $p \leq 0.05$ on log-transformed data). Total macroinvertebrate density does not include densities of *Dreissena* spp.

between years and other sediment zones. The lack of significant differences may be partially due to the small number of stations located in this zone.

The spatial distribution pattern of *Diporeia* was different between 1994 and 1997, especially in the low-depositional zone. In 1994, highest population densities were found at stations located along the northern shoreline and at the far eastern end of the lake (Fig. 3*a*). A contiguous zone of stations with population densities of <4 individuals·m^{-2} occurred along the southeastern shore between the Genessee River outlet and Mexico Bay. In 1997, the number of stations with population densities of <4 individuals·m^{-2} increased and these stations extended all around the lake. The exception was Station 62 near Toronto where densities were 1311·m^{-2}. In some areas, this depauperate zone extended as far as 26 km offshore and to a depth of 160 m and encompassed over 40% of the total area of the lake. The most severe effect was along the southern and eastern shoreline from the outlet of the Niagara River to Point Petre, where *Diporeia* were absent from samples collected in waters 140 m deep or less.

In 1994, there were large numbers (>5000·m^{-2}) of oligochaetes near the mouths of the Niagara (Station 201) and Genessee (Station 172) rivers and along Point Petre (Station 81, Fig. 3*b*). *Stylodrilus heringianus* was the most abundant oligochaete at these stations, accounting for over 30% of the total number. In 1997, the spatial pattern of Oligochaeta was similar to 1994 but with lower numbers in the low-depositional and high-depositional zones. Relatively high population densities of Sphaeriidae were present at stations located near the Kingston basin (Fig. 3*c*) in 1994. In 1997, the spatial pattern of Sphaeriidae was different from 1994 with fewer organisms near the eastern end of the lake.

Ponar grab samplers were used to estimate densities of dreissenids in 1997. Although dreissenids were present in 1994, the mussels were not collected or counted. The efficiency of this sampler for collecting attached mussels has

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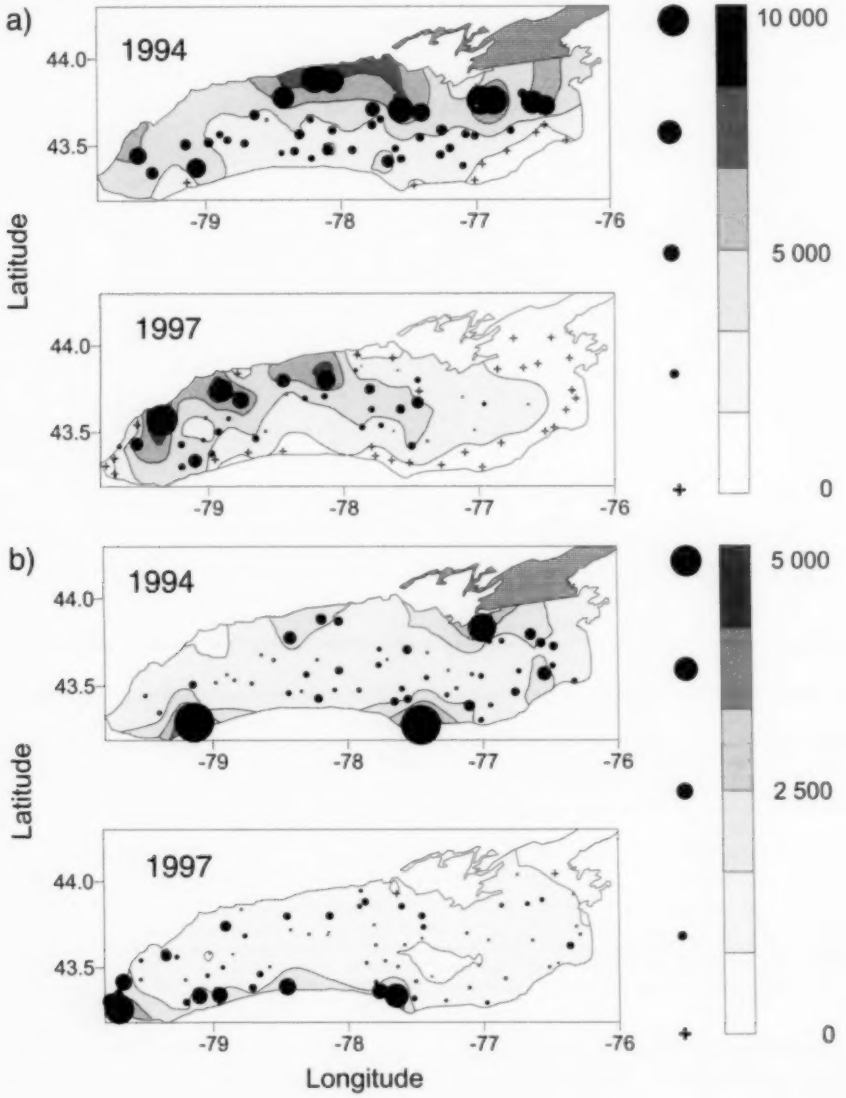
Fig. 3. Distribution and density (no.·m^{-2}) of (*a*) *Diporeia*, (*b*) Oligochaeta, and (*c*) Sphaeriidae in 1994 and 1997. Kingston basin was not sampled in 1994 (hatched area on map).



not been quantified so our results are a relative measure of population density and distribution. Mean densities of *Dreissena polymorpha* and *D. bugensis* in 1997 were highest in the low-depositional zone, intermediate in the transitional zone, and lowest in the high-depositional zone (Table 4). Neither species of *Dreissena* was abundant in waters deeper than 70 m. Stations along the south shore from the outlet of the Niagara River to Mexico Bay had the largest numbers of *Dreissena* in waters less than 40 m deep (Fig. 4). There were also large numbers of *Dreissena* near Port Hope, Ontario, and in the Kingston basin. Species richness (mean number of species·site^{-1}) in waters <40 m was greater at stations where *Dreissena* were present (10.1 ± 0.4 (mean ± SE), *t* test, $p < 0.05$) compared with stations where *Dreissena* were absent (6.4 ± 0.3). Several species of Naididae (*Chaetogaster diaphanus*, *Ophidonais serpentina*, *Stylaria lacustris*, *Vejdovskyella intermedia*), Tubificidae (*Potamothrix moldaviensis*, *P. vejdovskyi*, *Rhyacodrilus coccineus*, *Spirosperma ferox*), and Chironomidae (*Heterotrissocladius* sp., *Chironomus* sp., *Cryptochironomus* sp., *Paracladopelma* sp., *Paralauterborniella* sp., *Tribelos* sp., *Micropsectra* sp., *Tanytarsus* sp.) were more abundant or only found in stations where dreissenids were present.

Discussion

Our data suggest a large change in total and relative density of the macroinvertebrate community in Lake Ontario for areas of the lake deeper than 12 m. If *Dreissena* spp. are excluded from population estimates, our data indicate a major

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Fig. 3 (*concluded*).

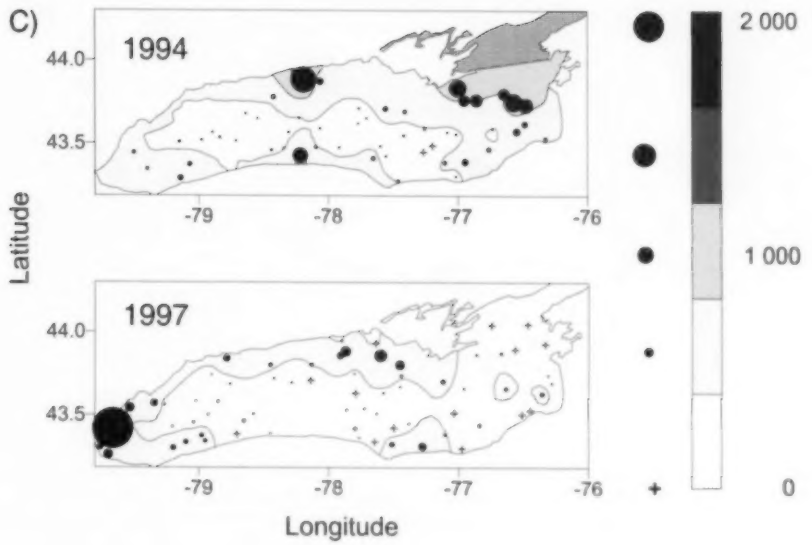

Table 4. Comparison between densities (no.·m^{-2}) of two species of *Dreissena* at three sediment zones.

	Low-depositional zone	Transitional zone	High-depositional zone
No. of stations	31	10	27
Dreissena polymorpha	357 (195) a	0.4 (0.4) bc	0 (0) bc
Dreissena bugensis	535 (174) a	2.2 (1.2) bd	0.3 (0.2) bd

Note: Values are means (±SE). Values followed by a different letter are significantly different (ANOVA followed by Tukey's test, $p \leq 0.05$ on log-transformed data).

decline in total macroinvertebrate densities between 1994 and 1997 in the low-depositional zone (12–88 m). Our results contrast those summarized by Nalepa (1991), who found no change in macroinvertebrate densities in shallow waters (10–35 m) between 1964 and 1988 and an increase in numbers of *Diporeia* in the Kingston basin. To allow comparisons to these earlier studies, we grouped our densities of *Diporeia*, Oligochaeta, and Sphaeriidae by the three depth intervals (Table 5) used by Nalepa (1991) for the two Lake Ontario surveys and tested for differences between years (analysis of variance, ANOVA, Tukey's test, $p < 0.05$) in each depth interval. These comparisons are important because the surveys of Hiltunen (1969) and Nalepa and Thomas (1976) were completed before the invasion of *Dreissena*.

In the shallow depth interval, *Diporeia*, Oligochaeta, and Sphaeriidae were significantly lower in 1997 compared with densities found in 1964 and 1972 (Table 5). Mean densities of *Diporeia* were at least 130 times greater in 1964 and 1972 than in 1997, while mean densities of Sphaeriidae and Oligochaeta were at least nine times greater in 1964 and 1972 than in 1997. The mean density of *Diporeia* in 1994 was 40·m^{-2} but the lack of statistically significant differences compared with earlier surveys is partially due to the small number of stations ($n = 4$) sampled in the shallow depth interval (10–35 m). In the intermediate depth interval, densities of *Diporeia* were significantly greater in 1972 and 1994 (2131–4537·m^{-2}) than in 1997 (1282·m^{-2}). Mean densities of *Diporeia* were much greater in 1964 (9077·m^{-2}) than in 1997 but the lack of statistical significance is also partially due to the small number of stations ($n = 2$) sampled in the intermediate depth interval (36–90 m) in 1964. In the deepest depth interval, results were mixed. Densities of *Diporeia* were variable but never significantly different between years. Densities of Oligochaeta and Sphaeriidae were significantly greater in 1964 than in 1972, 1994, and 1997 but there were no consistent trends after 1972. The spatial distribution of stations where *Diporeia* were absent in Lake Ontario sediments was similar in 1964 and 1972, i.e., the area extended along the south shore from the Niagara River eastward to Rochester, N.Y. (Hiltunen 1969; Nalepa and Thomas 1976). In 1997, we found the area where *Diporeia* was absent to have expanded to all areas around the lake in waters between 10–40 m and down to a depth of 140 m in the zone from the Niagara River eastward and northward to Point Petre, Ont. In addition to whole-lake surveys, Nalepa (1991) also provided a compilation of Lake Ontario benthic studies showing trends from a nearshore area (Kingston basin) of Lake Ontario before the invasion of *Dreissena*.

Between 1964 and 1988, *Diporeia* densities in the Kingston basin (28–35 m) of Lake Ontario were variable. Populations fluctuated between 3000·m^{-2} and 10 000·m^{-2} during the period of 1964 to 1984 and then rose to 13 900·m^{-2} by 1988. We found a mean population of *Diporeia* of less than 1·m^{-2} at six stations (stations 1–7) in the Kingston basin in 1997. Overall, the greatest change from 1964 to the present is a lake-wide decline in density of

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Fig. 4. Distribution and densities (no.·m^{-2}) of the mussel *Dreissena* spp. in 1997.

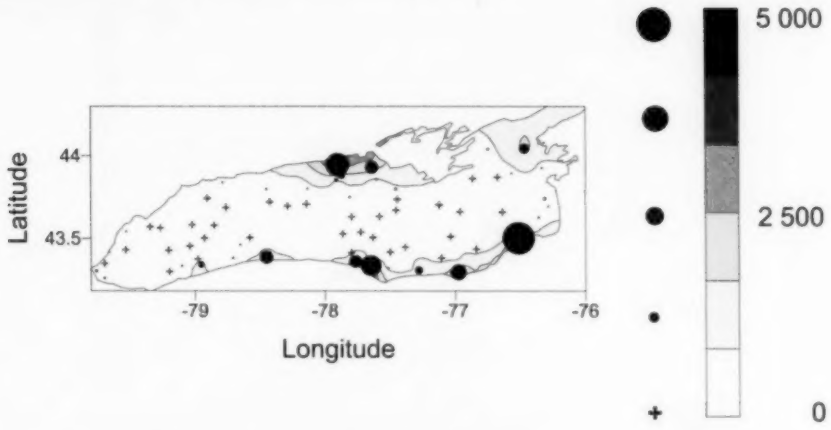

Table 5. Comparison between densities of three major benthic taxa found in 1964 (Hiltunen 1969), 1972 (Nalepa and Thomas 1976), 1994 and 1997 (present study) within three depth intervals.

	1964	1972	1994	1997
Depth 10–35 m				
No. of stations	14	22	4	16
Diporeia	2122 (815) a	2058 (598) a	40 (40) ab	15 (15) b
Oligochaeta	8536 (3203) a	9648 (3197) a	2476 (1186) ab	856 (280) b
Sphaeriidae	3223 (641) a	973 (275) b	113 (40) bc	109 (42) c
Depth 36–91 m				
No. of stations	2	13	12	25
Diporeia	9077 (891) ab	2131 (467) a	4537 (675) a	1281 (405) b
Oligochaeta	2607 (685)	2382 (816)	983 (210)	658 (100)
Sphaeriidae	3697 (262) a	107 (48) b	558 (100) a	233 (81) b
Depth >91 m				
No. of stations	8	20	35	27
Diporeia	1250 (356)	793 (141)	1677 (169)	1207 (162)
Oligochaeta	767 (128) a	353 (56) bc	405 (49) ab	174 (23) c
Sphaeriidae	244 (76) a	17 (12) b	65 (13) c	29 (7) c

Note: Values are means (±SE). Values followed by the same letter identify a significant grouping; values in bold are significantly different (ANOVA, Tukey's test, $p \leq 0.05$ on log-transformed data).

Diporeia down to depths of 140 m and lower densities of all three taxa in the shallow depth interval (10–35 m) and the low-depositional zone.

After the invasion of *Dreissena* spp. into the Great Lakes, ecosystem changes included loss of edible particles (e.g., phytoplankton) and subsequent decline of phytoplankton consumers, diversion of material from the open water to surface sediments as bivalve tissue and biodeposits, and subsequent increase of benthic resources in and around mussel beds (Strayer et al. 1999; Haynes et al. 1999). The negative impact of *Dreissena* spp. on *Diporeia* and other benthic macroinvertebrate species is attributed to its high filtering capacity and large population densities. Since their introduction to Lake Ontario in 1988 or 1989, *Dreissena* populations have expanded, reaching densities of 82 000·m^{-2} in Lake Ontario by 1998 (Schaner 1998). Increased water clarity and declines in phytoplankton density are directly correlated with increased densities of *Dreissena* in Lake Erie and Lake Huron (Fahnenstiel et al. 1995). Mean densities of planktonic diatoms were reduced by 80–92% compared with pre-mussel periods in Hatchery Bay, western Lake Erie (Holland 1993). Although the reduction of diatom biomass could be the result of other system changes (e.g., reduced phosphorus loadings), concentrations of phosphorus in the open waters of Lake Ontario have remained approximately 10 µg·L^{-1} since 1985 (Neilson et al. 1995).

Some of our results contrast those of Haynes et al. (1999), who found greater densities of Gammarid amphipods but no long-term changes in densities of oligochaetes and other species of macroinvertebrates at 7 m in cobble and artificial substrates after colonization by *Dreissena* in Lake Ontario. We found densities of three taxa to be lower in the low-depositional zone and shallow water interval (10–35 m) of Lake Ontario but species richness to be greater at stations where *Dreissena* were present. The diversion of algae, especially diatoms, from open waters to mussel beds and hard substrates and incorporated into bivalve tissue and biodeposits would benefit species that can use the shells for habitat and (or) biodeposits for food.

Changes in benthic populations in deep lakes have been

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Fig. 5. Comparison of the dreissenid densities calculated from regression analysis (dashed line) of 1997 data (open circles) and Bailey et al.'s (1999) estimates (solid line).

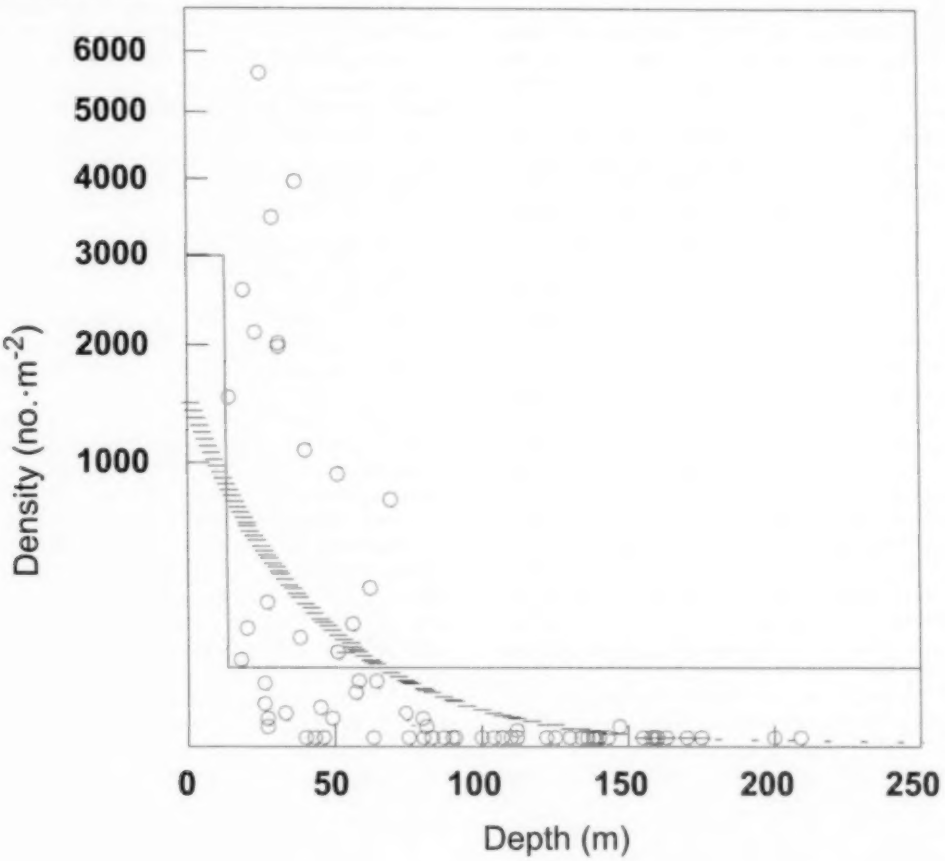

Table 6. Comparison of total turnover (days) at five depth intervals calculated with two dreissenid density estimates.

	Depth intervals (m)				
Density estimates from	0–15	0–30	0–45	0–60	0–75
Bailey et al. (1999)	0.1[a]	10.4	24.1	40.6	62.6
1997 Ponar grabs	0.4	2.4	7.4	19.3	51.4

[a]Total turnover is a summation of area-weighted average of the depth intervals (Bailey et al. 1999).

attributed to trends in water column productivity (Saether 1980) because the density of the benthos is directly related to the amount of food material settling from the water column. Earlier studies have shown that the amphipod *Gammarus fasciatus*, a shallow water species (<10 m), increased after invasion of mussels in southwestern Lake Ontario (Stewart and Haynes 1994; Haynes et al. 1999). The authors suggested that *Gammarus fasciatus* growth is facilitated by the additional food source from pseudofeces/feces deposited by *Dreissena* and additional habitat provided by mussel shells. Unlike *Gammarus*, *Diporeia* does not actively feed on *Dreissena* fecal pellets (Lydy and Landrum 1993). It has been shown that *Diporeia* is a discriminating feeder requiring a high quality food in the spring (diatoms) to complete its life cycle (Dermott and Corning 1988; Gardner et al. 1985). The inability of *Diporeia* to use *Dreissena* biodeposits as a food source and the reduction in its original food source may have contributed to its decline. The large decline in densities of *Diporeia* and other benthic macroinvertebrates in Lake Ontario was most pronounced near areas where dreissenids were most abundant and we postulate that these declines could have resulted from a redirection of energy from open waters to *Dreissena* beds.

Recent changes in densities of benthic macroinvertebrates after invasion of *Dreissena* are not restricted to Lake Ontario. Dramatic declines of *Diporeia* in deeper waters of eastern Lake Erie also coincided with colonization by dreissenids (Dermott and Kerec 1997). Mean densities of *Diporeia* declined from 1844·m^{-2} in 1979 to 218·m^{-2} in 1993; *Diporeia* was found at all 13 deepwater stations in 1979 but only five stations in 1993. Dermott and Kerec (1997) suggest that competition for diatoms between *Dreissena* and *Diporeia* led to the exclusion of amphipods from areas where *Dreissena* was abundant. In southern Lake Michigan, Nalepa et al. (1998) reported declines of *Diporeia*, Oligochaeta, and Sphaeriidae. Declines in oligochaetes and sphaeriids occurred uniformly throughout the

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nearshore region (<50 m), whereas declines of *Diporeia* were greatest in the southeastern region of the lake. The region where *Diporeia* declined more than 60% between 1980 and 1993 corresponded to areas where *Dreissena* was found in largest numbers. Nalepa et al. (1998) also hypothesized that the filtering activities of *D. polymorpha* were having a negative impact on *Diporeia*.

In Lake Ontario, *Diporeia* populations are declining even in areas with low dreissenid densities. *Dreissena* are not present in large numbers at depths greater than 40 m; greatest densities occur in waters less than 30 m. We hypothesize that filtering capacity of dreissenids may be enhanced and the reduction of *Diporeia* densities may be partially explained by the seasonal occurrence of isolated water masses in nearshore areas of the lake. The formation of a thermal bar in spring and early summer isolates nearshore waters from offshore waters. The progression of the thermal bar from areas near the shoreline to deeper waters (>100 m) usually begins in March and ends with the formation of the summer thermocline (Thomas et al. 1972). During the spring diatom bloom, diatoms shoreward of the thermal bar are exposed to warmer, nutrient-rich water and, because the waters are well mixed, are also exposed to the filtering activity of *Dreissena*, thus allowing *Dreissena* to intercept diatoms before they settle on deeper sediments. Scavia and Bennett (1980) used field observations and simulation models to demonstrate the relative importance of vertical mixing and in situ production to observed nutrient and algal biomass in Lake Ontario. During the spring, increased algal production occurs on the shoreward side of the 4°C isotherm (Stadelmann et al. 1974). The impact of dreissenids in the spring is directly related to their filtering capacity.

The seasonal impact of dreissenids was assessed by estimating their filtering capacity in comparison to volumes of water isolated by the thermal bar. Bailey et al.'s (1999) filtration model was used to calculate total turnover times (in days) at different depth intervals as

$$T = \sum_{i=1}^{S} [p_i \cdot v_i]/[f \cdot a_i \cdot x_i]$$

where p_i is the proportion of total bottom area in stratum i, v_i is the volume of stratum i, f is the filtering rate of an average-sized mussel (100 $mL \cdot h^{-1} \cdot mussel^{-1}$), a_i is the area of bottom in stratum i, and x_i is the density of mussels in stratum i. Total turnover times were estimated from dreissenid densities used by Bailey et al. (1999) and from Ponar data collected in 1997 (Fig. 5). To obtain total turnover times, we needed dreissenid densities at 1-m intervals; therefore, we used regression analysis of dreissenid densities on depth to find these values, i.e.,

$$\log (x_i) = 7.335 - 0.045 \cdot d_i$$

where d_i represents depth at stratum i. We found that total turnover times were similar for depth intervals less than 30 m but much smaller based on our dreissenid estimates at depth intervals between 30 and 75 m (Table 6). At depth intervals greater than 80 m, Bailey et al.'s (1999) total turnover times were substantially lower that those calculated from our estimates of dreissenid densities. It is clear that better estimates of dreissenid densities must be made before we can determine the true potential effect of dreissenid filtering on algal biomass in the spring. However, if our model and density estimates are fairly accurate, isolated water masses in Lake Ontario could be turned over in less than 10 days when the thermal bar is located at depth contours of 60 m or less.

The loss of *Diporeia* from large areas of Lake Ontario may be affecting fish production. Prey fish species such as slimy sculpin use *Diporeia* as a key food source in Lake Ontario (Owens and Weber 1995). Recently, there has been a decline in populations of this fish species in U.S. waters; estimates of densities of slimy sculpins have declined by 95% between the late 1980s and 1996 (New York Department of Environmental Conservation Annual Report 1998). Hoyle et al. (1999) found a sharp decline in both densities and body condition of lake trout and lake whitefish from the eastern region of the lake (Kingston basin) after 1990, which corresponds to the time that *Diporeia* were disappearing. In the past, the Kingston basin had produced the largest fish yields and supported a number of important commercial and sport fisheries. In 1997, a single specimen of *Diporeia* was collected from a total of 18 Ponar grab samples in the Kingston basin, where mean densities of *Diporeia* reached levels of 14 000·m^{-2} before the invasion of *Dreissena*.

In summary, the status of the benthic macroinvertebrate community of Lake Ontario has changed during the last 10 years. Densities of *Diporeia*, Oligochaeta, and Sphaeriidae have declined in soft substrate habitats of deep water, whereas dreissenid populations have increased in both hard and soft sediments. A zone of very low *Diporeia* density (<4 individuals·m^{-2}) extends as far as 26 km offshore and as deep as 200 m, encompassing over 40% of the total surface area of Lake Ontario soft sediments sampled in 1997. The reduction of benthos, especially populations of *Diporeia*, in the nearshore of Lake Ontario is expected to have a major impact on the upper trophic levels of the lake.

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Characteristics of a hydrothermal vent assemblage on a volcanically active segment of Juan de Fuca Ridge, northeast Pacific

Maia Tsurumi and Verena Tunnicliffe

Abstract: An eruption on Cleft segment, Juan de Fuca Ridge, in 1986 provided an opportunity to observe potential successional patterns in vent-animal colonisation. Other objectives were to describe the Cleft fauna, examine the distribution and abundance of selected taxa, and determine if the fauna reflected changes in water chemistry. Biological samples were tubeworm grabs collected by submersible, and visual data were still photographs and videos. Two years post eruption, there were extensive diffuse vents and 23 of the 44 species constituting the Cleft species pool were present. Five years post eruption, most low-temperature vents were extinct. High-temperature venting was maintained, and biological communities were reduced in visual extent. Four of the 44 species in samples from 1988–1994 accounted for over 90% of the individuals. Cluster analyses of species collected on tubes did not distinguish year or substratum differences, suggesting that a study of less than a year is necessary to document successional patterns at new vents. The Cleft subset of the Juan de Fuca Ridge species pool is likely adapted to episodic eruptive events on the decadal scale. Major changes in fluid chemistry did not result in detectable community changes other than habitat loss due to a decrease in dissolved sulphide availability.

Résumé : Une éruption sur la section Cleft de la dorsale Juan de Fuca en 1986 nous a permis d'observer les patterns potentiels de succession dans la colonisation par la faune des cheminées hydrothermales. Nos objectifs secondaires sont de décrire la faune de la section Cleft, d'étudier la répartition et la composition de taxons particuliers et d'établir si les changements faunistiques reflètent les variations dans la chimie de l'eau. Les données biologiques consistent en des récoltes de vers tubicoles à la benne réalisées par submersible et les données visuelles en des photographies et des films vidéo. Deux ans après l'éruption, il y avait un grand nombre de cheminées diffuses et 23 des 44 espèces du pool d'espèces de la section Cleft étaient présentes. Cinq ans après l'éruption, la plupart des cheminées à basse température étaient inactives. Il y avait des cheminées à haute température et, visuellement, les communautés biologiques y étaient réduites. Quatre des 44 espèces récoltées de 1988 à 1994 représentent plus de 90% de la densité totale. Des analyses de groupement des espèces récoltées sur les tubes ne permettent de distinguer ni les années ni les substrats, ce qui laisse croire qu'une étude au cours de la première année est nécessaire pour suivre la succession sur les nouvelles cheminées. Le sous-groupe d'espèces de la section Cleft du pool d'espèces de la dorsale Juan de Fuca est sans doute adapté à des éruptions sporadiques à une échelle de quelques dizaines d'années. Les variations importantes dans la chimie des fluides n'ont pas généré de changements décelables dans la communauté autres que des pertes d'habitat causées par une baisse de la disponibilité des sulfures dissous.

[Traduit par la Rédaction]

Introduction

The appearance of empty habitat after a large disruption or disaster provides an opportunity to study the behaviour of the community in the subsequent colonization process. Some organisms are well adapted to predictable disasters, such as forest fires (Halpern 1989) and wave action in rocky intertidal communities (Dayton 1971), and colonization may proceed relatively rapidly. Following massive landscape disruption, however, it may be decades before conditions are acceptable to a variety of organisms.

The hot-vent habitat sits atop a highly dynamic ridge crest, where both tectonic and volcanic activity may frequently disrupt the community (Juniper and Tunnicliffe 1997). While these communities are difficult to access and study systematically, repeat visits note substantial changes in vent assemblages (Fustec et al. 1987; Hessler et al. 1988). The process of sea-floor spreading includes the extrusion of new lavas to the sea floor above a magma chamber. Heat from the magma chamber induces hydrothermal circulation. An area prone to frequent eruptive activity usually has ongoing hydrothermalism and a local pool of vent species, such as are seen at the high spreading rate centres of the southeast Pacific (Embley et al. 1998). On the northern East Pacific Rise, thick microbial mats, large uncolonised surfaces, and even partly cooked animals indicated a recent eruption (Haymon et al. 1993). Within 1 year, vestimentiferan tube-

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worms were among the first to recruit, and many species appeared within 5 years (Lutz et al. 1994). On Juan de Fuca Ridge, even at a site where no adjacent communities were found, several species colonised eruption-induced venting in 1993 within 1 year (Tunnicliffe et al. 1997).

Vent animals rely on microbial production, which in turn, responds to chemical conditions in the venting fluids. A general model of post-eruption fluid evolution is presented by Butterfield et al. (1997): a change from a vapour-dominated phase of high heat flux to a brine-dominated phase in a later time of low heat flux. Sulphide peaks sometime after the initial eruption event, depending on the size of the system and longevity of the heat source. While temporal change in assemblages is observed at vents, the relationship of this change to chemical conditions is unknown. The current profile of post-colonization communities in the eastern Pacific includes: rapid appearance of macrofauna by larval recruitment, limited diversity with a changing dominance pattern in the succeeding years, very rapid growth of vestimentiferans, and diminishing microbial-mat coverage (Tunnicliffe et al. 1997; Shank et al. 1998).

The first sea-floor eruption recognized on mid-ocean ridges occurred on the northern Cleft segment of the Juan de Fuca Ridge. A huge bolus of hot water rose 800 m off the bottom in 1986 and disappeared within 1 month; a second megaplume was identified the following year (Baker et al. 1989). The plume character suggested a sudden expulsion of fluid during sea-floor extension. Subsequent side scan sonar and towed-camera surveys identified very young lavas and vigorous hydrothermalism (Fig. 1). These lava flows were in the form of pillow mounds and a southerly sheet (Embley and Chadwick 1994). Comparative bathymetric surveys constrained the appearance of the pillow mounds to between 1983 and 1987, but the sheet flow likely erupted before October 1982. The eruptive fissure lies mostly within the young sheet flow, with vents concentrated along this feature (Embley and Chadwick 1994; Fig. 2). Limited colonization by deep-sea fauna and poor sediment cover suggest an age not much older than this date (Milligan and Tunnicliffe 1994).

A bottom exploration programme began in 1987 with camera tows and continued in 1988 with submersibles. A description of the eruption scenario arose, as each succeeding year expanded the observations. Current interpretation is that the young sheet flow appeared in the 1970s. The 1986 and 1987 eruptions probably generated the northern pillow mounds, but also initiated venting on the young sheet flow and, in limited areas, on the pillow mounds. Embley and Chadwick (1994) implicate a magma chamber below the young sheet flow that fed the northern pillow mounds by dike injection. Subsequent cooling contracted the hydrothermal circulation to the sheet-flow area during the 1990s. The steady-state chronic plume subsided to a relatively low level from 1991 through 1997 (E. Baker, National Oceanic and Atmospheric Administration, Pacific Marine Environmental Laboratory, 7600 Sand Point Way NE, Seattle, Wash., personal communication). Mineralisation of the high temperature Monolith and Fountain sulphide structures (Fig. 2) likely occurred prior to the eruption of the northern pillow mounds, while Pipe Organ may be a product of this more recent event (Koski et al. 1994). The ages of the Aquarius and Marker 22 sites are unknown.

We consider 1986 to be the year that sheet flow venting was (re)initiated on north Cleft, following the precedent of Butterfield and Massoth (1994). These authors describe marked changes in vent-fluid chemistry in this system: they relate changes in water characters to a switch from vapour-dominated fluids in 1988 to brine-dominated fluids in 1990. They speculate that, following the eruption, widespread subsea-floor boiling discharged vapour- and sulphide-rich fluids first, while brines enriched in metals accumulated deeper in the hydrothermal system. With hindsight gained from eruptions studied elsewhere, and the pronounced water changes, we undertook to examine the biological collections for a successional pattern. Further objectives were to examine the distribution and abundance characteristics of selected taxa at these hydrothermal vents, and to determine if the fauna reflected the marked changes in water chemistry.

The data presented here extend post-eruption community observations from 3 (Tunnicliffe et al. 1997) to 7 years. Our study is the first attempt to describe vent succession quantitatively. Furthermore, this is the first study to illustrate the importance of including meiofauna in analyses of vent communities and to try to correlate evolution in water chemistry to changes in the faunal assemblage.

Materials and methods

Study site

Cleft is the 80 km long southernmost discrete segment of the Juan de Fuca Ridge (Fig. 1). The smooth shallow relief of the ridge flanks between 44°50′N and 45°02′N suggests a higher average rate of extensive volcanism than tectonism in the northern part (Embley and Chadwick 1994). Average depth in this area is 2200 m. North Cleft venting aligned on a trend of 020° (Fig. 2) spans about 11 km. The nearest vent communities occur on the southern end of the segment at 44°42′N (Fig. 1). Venting is not known on the next segment that overlaps to the north.

Sample collection

Samples were taken by submersible in the summers from 1988 to1994. The manipulators grabbed one or two samples of tubeworms, plus associated biota, which were placed in closable boxes. Organisms on the tubeworms were potentially lost during the transfer from manipulator to sample box. There was relatively little overlap in biological sampling sites, owing to bottom time limitations and other commitments. While some vents were revisited, the exact sampling spot could not be identified. A "vent," in this case, was either a concentrated area of flow through basalt over an area from 1 to 10 m^2 or venting through a mineralized chimney (sulphide) that usually had high temperature fluid orifices. It was not possible to sample a discrete bottom area on the rough hard terrain. Because the samples are vestimentiferan clumps, we present faunal statistics in relation to total surface area of the tubes.

Estimates of venting extent

Still photographs and videos were scored for areal coverage by indicator groups. Three categories of vent "indicator groups" were chosen for their distinctive appearance: bacterial mats, live tubeworms (*Ridgeia piscesae*), and "dead tubeworms." Dead tubeworms were tubeworm patches with no visible branchial plumes and extensive sediment cover (Fig. 3). A class system of scores from one to six represented total percent coverage by these indicators in images as estimated by eye: 1, less than 5%; 2, 5–20%; 3, 20–40%; 4, 40–60%; 5, 60–80%; and 6, greater than 80%. Figure 3, from a 1993 camera tow, had scores of 0 for bacterial-mat

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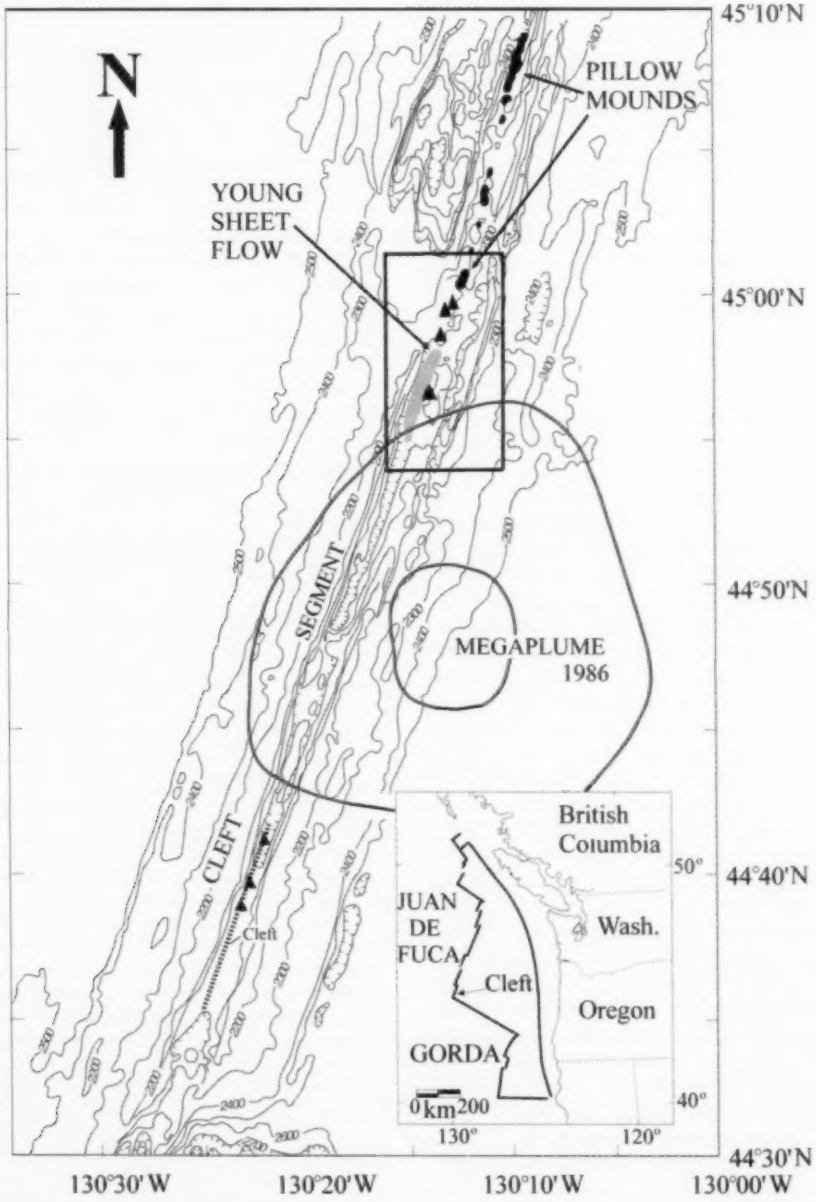
Fig. 1. Simplified Sea Beam map of Cleft segment, the southernmost segment of the Juan de Fuca Ridge (inset, bottom right). Contour interval is 100 m, except for the axial valley, where it is 20 m. High-temperature vents are indicated by filled triangles and the young sheet flow and northern pillow mounds by gray and black shading, respectively. The closest known venting is indicated on southern Cleft segment at 44°42′N. The megaplume contour shows the extent of the temperature anomaly associated with the megaplume. This temperature anomaly is used to detect and define hydrothermal plumes. Adapted from Embley and Chadwick (1994). The area shown in Fig. 2 is indicated (box on the ridge).



and live-tubeworm coverage and 5 for dead tubeworms. Geographic Information System (GIS) manipulations of spatial data allow visualization of venting changes and storage of information about venting conditions (Tsurumi 1998). The visual data cover more spatial and temporal ranges than the collections and, thus, allow a broader view of changes in venting. GIS manipulations were run in ArcView 2.1 from the database provided on-line (http://newport.pmel.noaa.gov/gis/data.html).

Sample processing

Samples were fixed in 7% seawater formalin at sea for transport to the laboratory for processing. All samples were sieved over a 1-mm mesh. The residue of one sample, Aquarius vent, was sieved over a 63-mm mesh. Samples were sorted and counted to species. Meiofauna were not included in analyses, but meiofauna from all samples are included in the species total.

Eighteen biological samples were selected for examination.

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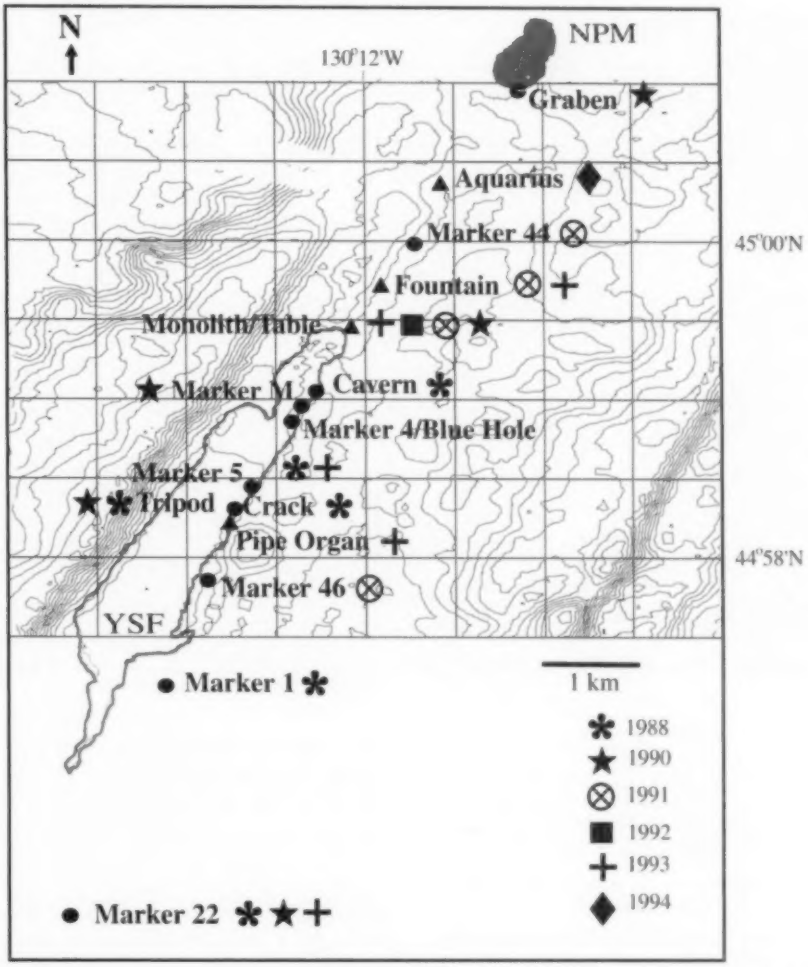
Fig. 2. Map of north Cleft with the sampling history for the region. The map was generated using the National Oceanic and Atmospheric Administration GIS database; detailed bathymetry is not available for the entire study area. Contour interval is 50 m. Triangles indicate high-temperature venting; YSF, young sheet flows; NPM, northern pillow mound.



Samples were culled to fit the following criteria: manipulator grabs of tubeworm bushes, specimens available to confirm any prior identifications, and tubeworm area measurable. Because numbers of species and individuals are not independent and our data set has a large number of rare species (species in low abundance), only collections of over 200 individuals were used. We set this minimum number of individuals to ensure the inclusion of at least some of these rare species in all analyses, in the hope that unrealistic clustering based on a few abundant species would not result. Subsamples of the 1988 specimens were used for other studies; thus, strict quantitative comparisons were not possible. Only presence–absence information was used from this year for cluster analyses and descriptive changes in venting. The 1988 data were adequate to present a relative assessment of the most abundant species and to include in overall diversity and rarefaction estimates. Missing species would lead to an underestimate of species diversity.

Analyses

Tubeworm area was measured to standardise sample size for comparison of the variable grabs. A digital image of all tubes in a sample was examined in the Bioscan Optimus 5.0 program (Biocybernetics). Plan area of each tube was converted to surface area by assuming a cylindrical shape. Species density was the number of species per tubeworm surface area in a sample.

Rosenzweig (1995) suggests that species number is the most appropriate measure of community diversity. We include the Shannon–Wiener (log base 2), Simpson's, and Margalef indices for comparison with other studies. Rarefaction computes the expected number of species ($E(Sn)$) in a random sample of n individuals (drawn without replacement from N individuals distributed among S species) as the sum of the probability that each species will be included in the sample (Hurlbert 1971). The slope of the rarefaction curve (i.e., the rate of addition of new species with additional individuals) reflects the richness and evenness of distribution of individuals among species. The rarefaction method was used to describe deep-sea diversity (Sanders 1968; Grassle and Maciolek 1992; Cosson-Sarradin et al. 1998). Rarefaction calculations were made in the BioDiversity Professional Programme 2.0 (McAleece 1997).

The standardized density data (number of animals/cm^2 of tubeworm area) were log-transformed. However, this transformation successfully normalized only two of the species chosen for analysis. No other transformations were able to normalize the data.

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Fig. 3. An example of a sea-floor photograph from a *ROPOS* dive on north Cleft. This image was taken at Marker 46 in July 1993. The sticklike objects are tubeworms. Note the heavy cover of iron-rich sediment and the moribund appearance of the animals. The area of this photograph is about 2 m^2. The score for indicator species is 0 for both bacterial mats and live tubeworms and 5 for dead tubeworms.

Number of individuals, species, and *R. piscesae* were log-normally distributed, as was *R. piscesae* area. Statistical tests were performed in SPSS and clustering in NTSYS 2.0 (Exeter Software); Jaccard and Bray-Curtis (flexible method; $\beta = -0.25$) were used.

Results

Changes in venting

Towed-camera images of the Marker 22 area revealed vigorous vent communities with large tubeworms in 1987. Marker 22 was south of the young sheet flow, and venting in this area may have predated the 1986 eruption. Dives in 1988 on the southern part of the young sheet flow found diffuse vents with extensive bacterial mats, healthy tubeworms, shimmering flows to 25°C, and flocculated white particulates. On the cleft walls at Cavern vent, gelatinous deposits up to 10 cm thick were embedded within microbial mats. Some Cavern tubeworms were over 180 cm in length in flows of 21°C. Tripod vent had temperatures up to 59°C. In 1990, both camera tows and submersible dives recorded reduced venting and animal coverage. Iron-rich sediments smothered several marked sites, such as Tripod and Marker 22. A few sites maintained reduced activity (Table 1) but extensive mats and floc were gone. Northern sulphide structures were discovered this year (1990). By 1991, 5 years post eruption, most low-temperature vents were extinct; the highest temperature was 30°C. In the next 3 years, most of the 1988 sites were revisited—only 2 of 10 had any residual venting. Extensive fields of tubeworms were sedimented and decaying. The four high-temperature chimneys maintained activity, albeit reduced, in this period; Monolith was sampled for 5 successive years and dropped from 324 to 295°C.

Table 2 presents average percent coverage of indicator species on photographs for 1988, 1990, and 1991. Coverage by bacterial mats and tubeworms was greatest in 1988 and decreased markedly in 1990 and 1991. Average percent coverage of dead tubeworms increased from 1988 to 1991. Even these remnants disappeared in later years.

Taxonomic character

Eighteen collections of *R. piscesae* grabs yielded 140 418 individuals on the tubes. There were 28 species averaging over 1 mm in adult size (Table 3) and another 16 meiofaunal species (Table 4). Seventeen species constituted new records for this part of the Juan de Fuca Ridge, another nine were possibly new to science and (or) vents, three were newly described species, and two were new species records at vents. Across all samples, 16 out of a total of 44 species were meiofauna (34.8%). The remaining 28 species were macrofauna. In the one sample sorted carefully for meiofauna on a 63-μm sieve, 10 of 27 species were meiofauna.

Taxonomic diversity

Results for selected diversity indices were as follows: Margalef's ($S - 1 / \log N$) was 8.68; Shannon–Wiener (H') was 1.73; and Simpson's ($1 - D$) was 0.69. The shape of the

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Table 1. Observations of vents visited from 1987 to 1995 by submersible and, in 1987 and 1989, by towed camera.

Vent	Substratum	1987	1988	1989	1990	1991	1992	1993	1994	1995
Marker 22 area	Basalt	V	*V		*V			*D		E
North of Marker 1	Basalt		*V					(D)		
Marker 46	Basalt					*V		D		
Tripod area	Basalt		*V		D	D			E	E
Crack	Basalt		*V						E?	E
From Crack to Marker 4	Basalt		*V		E			*V	D	
Cavern – Marker M	Basalt		*V		*V			(D)	D–NV	
Marker 44 area	Basalt					*D			D	
Graben	Basalt			NV	*NV	NV				
Northern pillow mounds	Basalt			NV		NV				
Pipe Organ vent	Sulphide					V			V	V
Monolith–Table vents	Sulphide				*V	*V	*V	*V	V	V
Fountain vent	Sulphide				V	*V		*V		
Aquarius vent	Sulphide								*V	

Note: An asterisk (*) denotes a biological sample; V, vigorous community; NV, not vigorous community; D, dead tubeworms, iron staining, and maybe a few lingering animals; E, extinct, no signs of any venting or dead animals. Symbols in parentheses indicate that the marker was not located, but that worm fields in the vicinity were examined.

Table 2. Still images from the submersible *Alvin* were scored for cover estimates by three vent "indicator groups" over 3 years; estimates for each indicator are presented as the average percent cover of indicator species from all the images with indicator species.

Indicator	1988	1990	1991
Microbial mats	22.5	3.90	5.84
Live tubeworms	6.12	3.05	1.05
Dead tubeworms – sediments	0.00	15.5	16.2

Cleft rarefaction curve differed markedly from three other studies of deep-sea fauna (Fig. 4). The shallow slope reflects the great unevenness in species distribution among individuals and the low richness at Cleft.

Dominance and dispersion

The four most abundant macrofaunal animals were two gastropods and two polychaetes that made up over 90% of the enumerated collections (Table 3). The three next most abundant taxa were an order of magnitude lower than the top four (totalling 7.5%). These were followed by 16 species, each representing much less than 1% average relative abundance. The limpet *Lepetodrilus fucensis* was the first, second, or third most abundant animal in 9 out of 10 samples used and, in the 10th, was not found (Fig. 5). Similarly, *Depressigyra globulus* and *Amphisamytha galapagensis* were dominant in most collections.

The occurrence pattern of the polychaete *Paralvinella pandorae* differed. Although information for our 1988 collections was not complete for all species, the abundance of this animal could be assessed. *Paralvinella pandorae* represented 70% of the >18 000 animals identified in this year; it occurred at all sites, constituting 20–98% of the community. In 1990, 88% of the >70 000 animals identified were individuals of this species. However, all but 10 specimens came from a single site: Monolith. In the combined 1991–1994 collections, fewer than 1% of the >22 000 animals collected were *P. pandorae*. The four most abundant species were also the most well dispersed (Table 3), being found at 10 or 11 of the 14 sampled sites. Of the macrofaunal species listed in Table 3, 46% were found at only one, two, or three sites. Three species were seen at only one site in 1988 and never again: *Temnocinclis euripes*, *Paralvinella dela*, and *Thermanemertes valens*.

Substratum

Ridgeia piscesae area was not correlated with number of species. Organism abundances were not affected by the type of substrate occupied by *R. piscesae* (basalt or sulphide).

A wide range of animal densities was encountered in the 1990–1994 samples: from 0.01 to 3.34 animals/cm^2 in one 1993 sample. Limpets alone in the 1993 sample reached nearly 3 animals/cm^2; at about 0.5 cm long, these animals crowd a desirable substratum by stacking and, apparently, suspension-feed. The base of Monolith sponsored high densities of *P. pandorae* (2.70 animals/cm^2), where the deposit-feeding polychaetes built sheaths overlapping each other. The Fountain sample with fat white tubeworms had the lowest density, at 0.01 animals/cm^2, and was also depauperate in species (eight species among 728 individuals).

Community similarity

Data from 16 collections from 1988 to 1994 were reduced to species presence–absence; singleton occurrences were converted to zeros. The Jaccard similarity coefficient indicated two major clusters (Fig. 6*a*), but sites did not cluster by year. The major division separated lower- from higher-diversity groups. The only consistent cluster comprised the four Monolith and Table samples in 1990 (Table is only a few metres from Monolith); in 1992, Monolith differed enough to move to another part of the tree. Marker 22 and Aquarius were the most widely separated sites, yet the most speciose.

Species associations

Possible associations among macrofaunal species were examined with cluster techniques. Only species that occurred at more than two sites were used. Figure 6*b* suggests three major clusters based on presence–absence data. Two-way analyses of associations were run (Kendall's Tau and Pearson's

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Table 3. Percent relative abundance of macrofaunal species in 10 collections from 1990 to 1994.

		No. of sites present (out of 14)	% average relative abundance
Species from comparable samples[a]			
Gastropoda	*Lepetodrilus fucensis*	10	35.558
	Depressigyra globulus	11	18.656
Polychaeta	*Paralvinella pandorae*	10	18.248
	Amphisamytha galapagensis	11	18.066
	Paralvinella sulfincola	6	4.352
	Paralvinella palmiformis	8	1.857
	Parougia wolfi	8	1.224
	Lepidonotopodium piscesae	9	0.582
	Branchinotogluma sp.	10	0.321
Gastropoda	*Provanna variabilis*	6	0.226
Polychaeta	*Nicomache venticola*	4	0.201
	Hesiospina vestimentifera	3	0.198
Arachnida	*Ammothea verenae*	5	0.154
Aplacophora	*Helicoradomenia juani*	1	0.096
Polychaeta	*Levensteiniella kincaidi*	7	0.062
Ostracoda	*Euphilomedes climax*	4	0.047
Polychaeta	*Opisthotrochopodus tunnicliffeae*	3	0.046
	Prionospio n.sp.	2	0.033
Gastropoda	*Clypeosectus curvus*	2	0.030
Amphipoda	Amphipod spp.	4	0.028
Polychaeta	*Ophryotrocha globopalpata*	2	0.008
	Protomystides verenae	2	0.004
	Hesiodeira glabra	1	0
Additional species from other samples[b]			
Polychaeta	*Paralvinella dela*	1	—
Amphipoda	Sp. near *Valettiopsis*	1	—
Scyphozoa	*Lucenaria* n.sp.	1	—
Gastropoda	*Temnocinclis euripes*	1	—
Nemertea	*Thermanemertes valens*	1	—

[a]Samples with consistent collection–retention techniques; the value is the average over these samples; a "site" is a discrete geographical location of a vent.

[b]Additional macrofaunal species recorded from samples not used for quantitative comparison; these species were found in samples that were subsampled, and so percent average relative abundance could not be calculated.

correlation) on the densities of the nine most abundant species in the 1990–1994 samples selected for analysis. Associations among *A. galapagensis*, *L. fucensis*, and *D. globulus* were significant ($p < 0.05$), as was the association between *Branchinotogluma* sp., and *Provanna variabilis*. Densities of *Paralvinella palmiformis* and *D. globulus* were significantly associated as were those of *P. pandorae* and *Paralvinella sulfincola*. All associations were positive.

Discussion

Cleft segment may be subject to cyclical and periodic eruptions on the decade to century scale, owing to magma surges and declinations (Lichtman and Essen 1983; Kappel and Ryan 1986; Baker et al. 1989). The 1986 eruption, the colonisation of new vents, and the eventual loss of tubeworm habitat likely represented a typical cycle for the vent communities in this geophysical setting. By 5 years post eruption, most low-temperature vents were extinguished and, by 1994, all were gone. Areas of focused flow at high-temperature chimneys remained active, although diminished in extent and temperature (Butterfield and Massoth 1994). These sites may sustain focused flow for much longer (Koski et al. 1994) and, thus, may provide a ready supply of propagules to populate periodic venting during episodes of crustal accretion.

The following changes in diffuse fluids were described from Cleft during 1988–1991: chloride nearly threefold, sulphate up fivefold, sulphide from 6 mmol·kg^{-1} to nearly zero, iron up 30-fold, and manganese up threefold (Butterfield and Massoth 1994). Where diffuse vents survived, temperatures were generally lower. The shifts were related to a change from vapour-dominated fluids still undergoing phase separation to brine-dominated fluids. On a gross scale, these fluid changes had a great influence on the fauna as a direct result of the loss of available dissolved sulphide and smothering by iron-rich precipitates. Sulphide depletion likely occurs owing to subsurface chemical and microbial oxidation; a similar abrupt drop in the heat to sulphide ratio was recorded at the CoAxial vents (Butterfield et al. 1997). Among the diffuse vents that remained, however, animals showed no detectable responses to fluid changes during the vapour to

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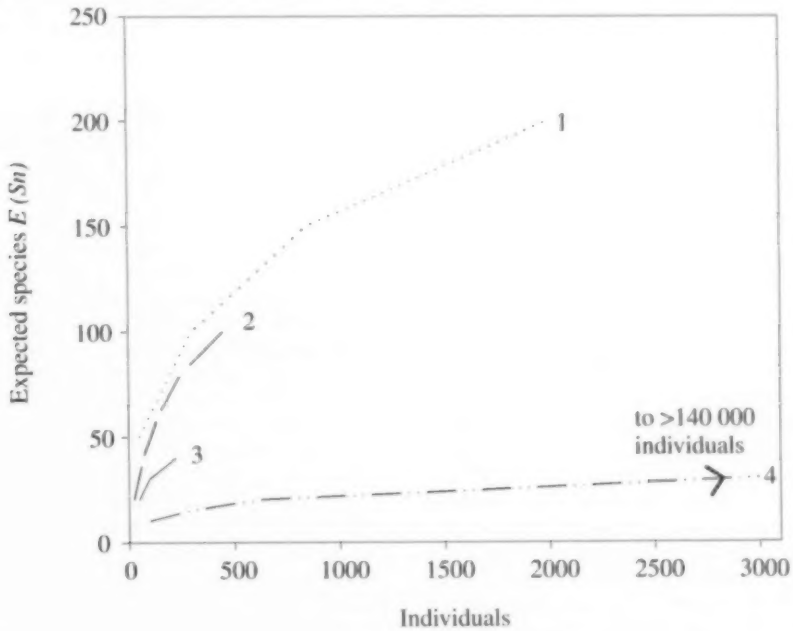
Table 4. Listing of all meiofauna recovered from Cleft samples, together with the relative abundance (%) of these taxa in one carefully sorted sample.

		Species	No. of individuals in the Aquarius sample
Acarina	Family Halacaridae	*Copidognathus papillatus*	80
Copepoda	Family Pseudocalanidae	*Clausocalanus lividus*	0
	Family Ameridae	Unknown	483
	Family Diosaccidae	Unknown	1636
	Family Tisbidae	Unknown	40
	Family Ectinosomidae	Unknown	20
	Family Thalestridae	Unknown	20
	Family Tisbidae	Unknown	0
	Siphonostomatoida	*Aphotopontius forcipatus*	0
		Benthoxynus spiculifer	568
		Stygiopontius quadrispinosus	617
		Collocherides brychius	0
	Misophrioida	*Misophriopsis longicauda*	0
Ostracoda	Myodocopida	Unknown spp.	780
	Podocopida	*Microloxoconcha* sp. (?)	60
Nematoda		Unknown spp.	0

Note: The unknown copepods are all harpacticoids. The meiofaunal component in the Aquarius sample constituted 27% of all individuals and 44% of the taxa recovered.

Fig. 4. Rarefaction curve of the north Cleft data, as well as curves reproduced from three other deep-sea studies. The curves of the nonvent fauna are of similar shape, indicating a similar pattern of species diversity and evenness. Curve 1, off New Jersey and Delaware, 1500–2500 m (Grassle and Maciolek 1992); 2, tropical northeast Atlantic, 1700–4600 m (Cosson-Sarradin et al. 1998); 3, Newport Submarine Canyon, Calif. 31–500 m (Maurer et al. 1995); 4, north Cleft, 2200 m.



brine transition. Diffuse fluids are much diluted by subsurface seawater leakage. Pronounced hydrothermal changes in this milieu may have little effect.

The north Cleft fauna differed in one notable character from the other studied sites on Juan de Fuca Ridge: the species list of 44 species was shorter. For comparison, 61 species were found in one Endeavour segment field among 75 000 individuals and 62 species on Axial Volcano among 530 000 plus individuals (V. Tunnicliffe, unpublished data). There were no species endemic to the Cleft community (species that were newly recorded from Cleft are now known from other sites). The fact that the north Cleft fauna was a

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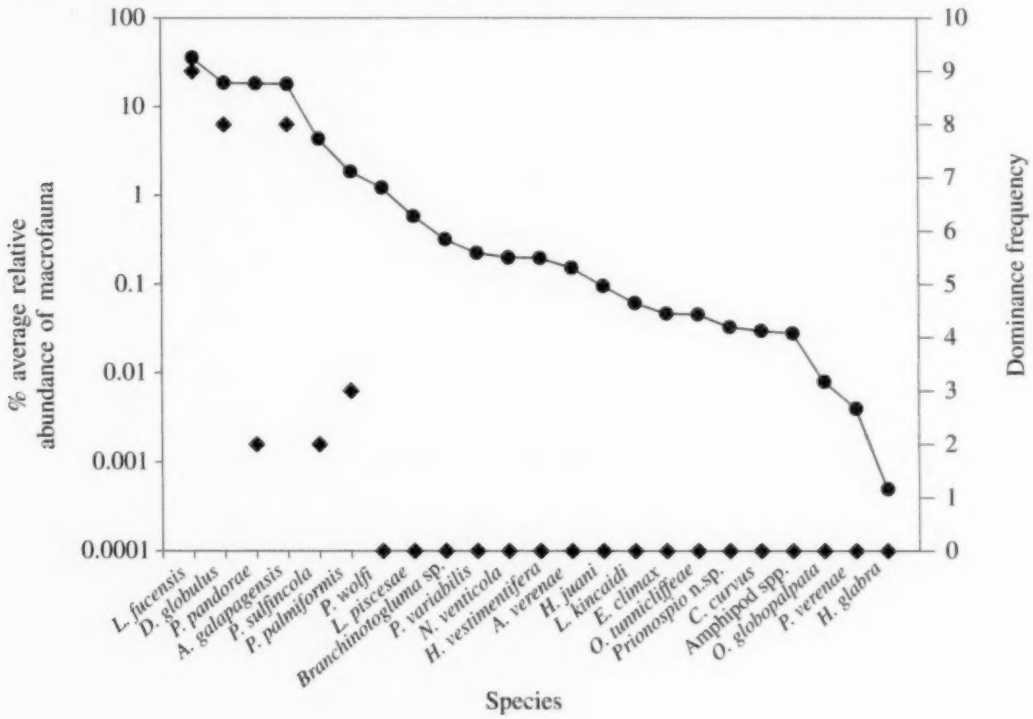
Fig. 5. Rank abundance of the macrofauna in 10 samples (circles). The frequency of their dominance in the samples is also recorded (diamonds). Rank abundance was calculated as the percent average relative abundance of each species across all 18 samples. Dominance frequency is the number of times a species was among the three most abundant species in each sample.



subset of the entire Juan de Fuca Ridge species pool probably reflects the frequency of habitat turnover on this segment. In the larger context, Juniper and Tunnicliffe (1997) linked spreading rate, frequency of magmatic events, and habitat unpredictability, to partly explain differences in regional vent diversity around the world. North Cleft is a segment-scale expression of the same phenomenon. This assemblage may represent a "rapid response fauna" for the Juan de Fuca Ridge. The paucity of species on Cleft compared with the Juan de Fuca Ridge species pool may reflect the demands of a habitat that is continually turning over on the scale of a decade or less.

In 1988, north Cleft communities looked much like those at the post-eruptive vents on CoAxial segment (Tunnicliffe et al. 1997) and Axial Volcano (Tsurumi et al. 1998) at 2 years after eruption: (*i*) extensive microbial mats and local areas with thick sacs of gelatinous polysaccharides; (*ii*) large fat *R. piscesae*; (*iii*) large, abundant, and well-dispersed *P. pandorae*; and (*iv*) flocculated particulates in cracks in the basalt. Shank et al. (1998) reported the presence of microbial mats, tubeworms well-established by the second year post eruption, and an increase in species richness by the third year post eruption at 9°N on the East Pacific Rise. From 1988 to 1990, the coverage by microbial mats decreased, tubeworms became long and skinny, *P. pandorae* became smaller and localized, and flocculated particles disappeared. Dead tubeworms smothered in sediment were abundant and, by 1991, most diffuse habitat was lost.

However, despite dramatic shifts in vent visual and areal character, collections did not group by year in the cluster analysis. By 1988, we had already missed sampling the initial stages of colonisation. Within 2 years, most of the Cleft fauna had colonized, probably seeded from the chimneys. Subsequent vent deaths were so quick that the limited sampling of 1990 was insufficient to detect senescence. Small plumbing changes initiated one site near Marker 4 (Blue Hole) in 1993, which clustered with the 1988 vents; we cannot assume that heat flux diminished consistently. We show some geographic consistency, as collections from the same site in the same years clustered. The vestimentiferan *R. piscesae* is a rapid colonizer (Tunnicliffe et al. 1997) and, by 2 years post eruption, formed extensive bushes, with some individuals nearly 2 m long. The tubes of *R. piscesae* create a three-dimensional structure for other vent animals to inhabit. For some vent animals, the presence of tubeworms and the microhabitats created by their physical structure may be the main prerequisite for colonisation. Even when there is no measurable sulphide or temperature anomaly at a vent, some vent fauna persist among the dead or dying tubeworms, perhaps until the tubes themselves are gone (M. Tsurumi, unpublished data).

Two species may be very early colonizers. The nemertean *Thermanemertes valens* was an initial coloniser after the CoAxial eruption. It is otherwise known only at vents 1–2 years post eruption on Axial (V. Tunnicliffe, unpublished data) and at Cleft in 1988. It may be out-competed for resources by late-arriving species. The scyphozoan *Lucenaria* n.sp. was found in small numbers at one site in the second year after

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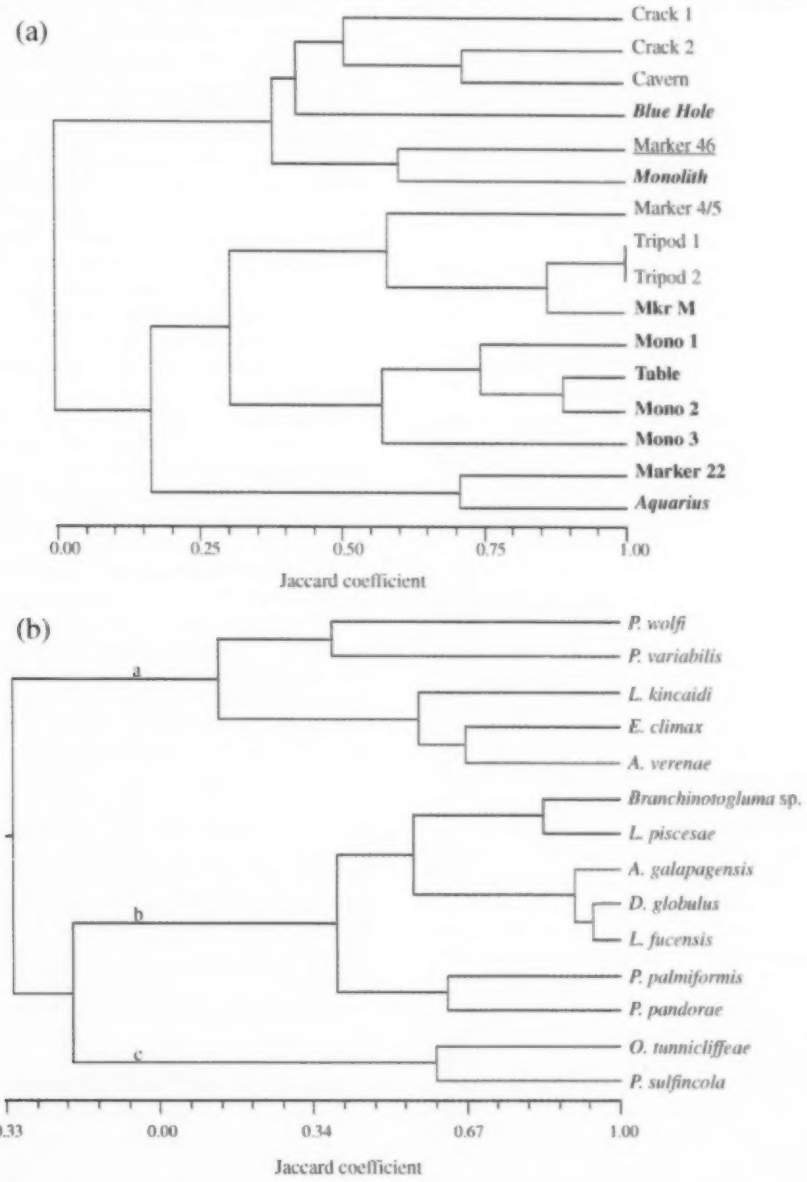
Fig. 6. (*a*) Cluster diagram of a similarity matrix generated by the Jaccard Coefficient, based on presence–absence of species in *R. piscesae* tube collections. Sample year is designated by font: normal indicates 1988; bold, 1990; underlined, 1991; bold–italic, 1992–1994 inclusive. (*b*) Cluster diagram of species associations generated by the Jaccard Coefficient, based on the presence–absence of species in *R. piscesae* tube collections. Cluster "*a*" species are associated with low-temperature habitats, cluster "*b*" species with warmer habitats, and cluster "*c*" species with the hottest environment. Mono 1, 2, and 3 are three different samples from Monolith collected in 1990. Samples from Pipe Organ and Graben vents were too compromised to be used in the cluster analysis.



eruption on CoAxial and was found in similar numbers at one site 4 years post eruption at Cleft.

Most measures of species diversity from hard-substratum vent sites are restricted to species lists and are statistically not comparable. Van Dover and Trask (2000) used a standardised sampling volume to compare mussel bed diversity between a Mid-Atlantic Ridge vent site and the intertidal. We chose tubeworm area to make north Cleft samples comparable, as tubeworms provided most of the three-dimensional habitat for animals living at low-temperature basalt venting and also covered substantial amounts of sulphide chimneys (Sarrazin et al. 1997; Sarrazin and Juniper 1999).

The Shannon–Wiener index (H') for Cleft (1.73) was comparable with that for the vent mussel bed (2.31, when converted to log base 2) studied by Van Dover and Trask (2000). The rank-abundance curves for these mussel beds

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and for Cleft were similar in shape, with five or fewer species accounting for 90% of the total number of individuals. H' was much lower for Cleft than for deep-sea soft sediments (converts to 19.3 in base 2) (Grassle and Maciolek 1992). The rarefaction curve for Cleft was markedly different from selected curves reported in deep-sea sediment studies. Grassle and Maciolek (1992) found very high evenness of fauna in deep-sea sediments; Maurer et al. (1995) found less evenness, but dominance was still spread over 24 taxa rather than 5. Areas subject to frequent physical disturbance in the deep sea have reduced species diversity and are more like shallow-water communities, because they have species that are numerically dominant (Grassle and Maciolek 1992). The unevenness of the vent environment may be due to habitat that is both unpredictable and frequently disturbed.

The limpet *L. fucensis* had the highest dominance frequency, attaining densities of up to 3 animals/cm^2 at Blue Hole. Sarrazin et al. (1999) reported that *L. fucensis* was the densest species (10.8 animals/cm^2) on an Endeavour sulphide chimney. This limpet will stack vertically on any substratum and may be suspension-feeding. De Burgh and Singla (1984) reported bacterial colonisation and endocytosis on the gill of this species, and stacking may be a way of bringing nutrients to its bacteria from the water column. They suggested that the animal is both filter- and deposit-feeding. If *L. fucensis* has multiple food-gathering strategies but is not dependent on a symbiont, it may be able to live in a variety of vent habitats. Although nothing is known about the reproduction and dispersal of *L. fucensis*, *Lepetodrilus elevatus*, a congener, is recorded as having had region-wide synchronous settlement on the East Pacific Rise (Mullineaux et al. 1998).

Although it was the third most abundant species, *P. pandorae* dominated only two samples. Although this species appears to be dependent on *R. piscesae* for a substratum, there was no apparent correlation between its abundance and the surface area of *R. piscesae*. The dispersion pattern of *P. pandorae* changed markedly over 5 years. The phenomenon described by Tunnicliffe et al. (1997) was seen here: a body-size shift from large to small adults in later post-eruptive years. This feature, combined with decreasing site occupation, suggested that habitat suitability, not dispersal capability, may limit this species. The cause is not clear but may involve competition with *P. palmiformis* and *A. galapagensis*, species that share a similar habitat attached to tubeworms.

Two polynoid polychaetes, *Branchinotogluma* sp. (the species designations are under revision; S. Hourdez, 208 Mueller Laboratory, Pennsylvania State University, University Park, Penn., personal communication) and *Levensteiniella kincaidi*, were very well dispersed, despite low abundances. Both belong to a small subset of species (which includes *A. galapagensis* and *Hesiospina vestimentifera*) shared with the East Pacific Rise. *Lepidonotopodium piscesae*, another polynoid polychaete, was also well dispersed. A generalist–opportunist life-history strategy may allow early entry into the vent sites and (or) persistence in a specialised niche with a low carrying capacity. Species with low local abundances may avoid extinction if they are widespread.

We did not see the distinct separation of chimney and basalt communities found by Jollivet (1996) on northern East Pacific Rise. While there was some separation of basalt- and sulphide-based assemblages, the location on a chimney appeared to be important. On Juan de Fuca Ridge, different flow and (or) temperature regimes foster different assemblages (Sarrazin and Juniper 1999), and there is considerable overlap in species presence both among these groupings and in basalt-hosted assemblages. More work is required to define the habitat characters of the three clusters, but the groupings agreed with our observations of relative temperature.

Limitations on larval dispersal were not evident at the scale of the north Cleft community (about 20 km), as the same species occurred at the geographic extremes; the samples with the highest diversity were farthest apart. Because neither geographic location nor year of collection appeared to influence clusters, local habitat-scale features (sulphide supply, food availability, and competition) may determine species composition. The wide ranges in both density and diversity may reflect the range of microhabitats available to the animals. Thus, the cooling of Monolith may have changed the 1992 habitat to resemble diffuse conditions rather than the conditions at Monolith in 1990. That Marker 22 was dissimilar to all vents but Aquarius may be due to its pre-eruption existence.

An oft-noted pattern in ecology is that the most abundant species also tend to be the most widely distributed (Hanski 1982). There was a positive relationship between average local densities and geographical distributions of species on Cleft. Although the underlying causes for a positive abundance–range relationship are not understood, abundant and widespread species may be those able to use a broader range of resources (Brown 1984). A few species on north Cleft, such as *Branchinotogluma* sp., had low local densities, yet wide distributions. Hanski et al. (1993) suggest that species with low local abundances and wide distributions will have low local growth rates in relation to their emigration rate, and will also have high mortality during dispersal. Species with both a small range and low local abundances should be at high risk of extinction. However, the 12 species with the narrowest distributions on north Cleft were also the ones with the lowest abundances. The establishment of a species at a particular vent site is a balance between local probabilities of extinction and colonisation. Species with a narrow distribution may maintain their metapopulations by having a very high colonisation rate. Further work on other sections of the Juan de Fuca Ridge is needed, to see whether this pattern is repeated.

The inconsistent biological sampling at Cleft had at least two consequences. It reduced the number of samples available for statistical analyses and only allowed inclusion of macrofauna in these analyses. Because of the overwhelming dominance of four species, excluding meiofauna, probably did not distort the overall community character. However, without the meiofauna, we do not have a complete picture of diversity and (or) taxonomic character. In the one sample sorted to the 63-mm size-class, over a third of the species were in the meiofauna, which included mites, nematodes, crustaceans, and juveniles of most species. Sorting to meiofauna may alter our idea of vent-community organisation and may be important in distinguishing senescent from nonsenescent assemblages (M. Tsurumi, unpublished data). Small crustaceans that are a major component of nonvent deep-sea fauna may be more prevalent and diverse at vents

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than previously recognised (M. Tsurumi, unpublished data). Many crustaceans found at vents may not be vent endemic. For example, *Clausocalanus lividus*, a common mid- to deep-water calanoid copepod, is reported here for the first time at vents (F. Ferrari, Invertebrate Zoology, Museum Support Center, National Museum of Natural History, Smithsonian Institution, Washington, D.C., personal communication).

In summary, by 2 years post eruption, more than half the Cleft species pool was present on north Cleft. Only an investigative response in less than a year can map the pattern of arrival and replacement at new vents. An increase in diversity was seen neither in subsequent years nor at high-temperature chimneys that had likely been venting before the 1986 eruption. The north Cleft subset of the Juan de Fuca Ridge species pool is likely adapted to episodic and frequent eruptive events on the segment. Major changes in fluid composition at vents, because of a shift from vapour- to brine-dominated fluids, resulted in no detectable community changes other than habitat loss due to a large decrease in dissolved-sulphide availability. Habitat loss from 1988 to 1991 was greater than 70%.

On the scale of the 20 km of the north Cleft vents, larval interchange homogenised the character of the community. This homogenisation made it difficult to distinguish temporal, spatial, or substratum differences. Cleft vents had low diversity and evenness, with 90% of the individuals being of four species. These four dominants are responsible for the community character, although small-scale habitat variability was likely important in determining exact assemblage compositions. The extreme unevenness of the Cleft community reflects the dominance pattern in frequently disturbed habitats, such as the rocky intertidal and areas of the deep-sea subject to sediment-eroding benthic storms. Assemblage diversity changes markedly when animals under 1 mm are considered: one-third of the species found on Cleft were meiofauna.

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Diatom-inferred dissolved organic carbon reconstructions provide assessments of past UV-B penetration in Canadian Shield lakes

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Abstract: Paleolimnological approaches have provided strong inference models for lake-water acidity and metal concentrations in Sudbury-area lakes, but calibration data have not yet been explored for inferring lake-water dissolved organic carbon (DOC). A review of available limnological data provided DOC values for 80 of our 105 calibration lakes, allowing us to examine the relative importance of DOC in determining the distribution of diatom assemblages in Canadian Shield lakes, such as those in the Sudbury region. Canonical correspondence analysis (CCA) indicated that lake-water DOC explained a significant proportion of variation in the diatom data. Our weighted-averaging DOC model showed a strong relationship (r^2 = 0.63) between measured and inferred DOC. Using this model, we reconstructed historical DOC concentrations in three Sudbury-area lakes, and then estimated temporal changes in UV-B penetration. These reconstructions showed that lake water DOC concentrations and underwater UV-B penetration have changed markedly in two of the three lakes modeled. The close correspondence between inferred and measured DOC for the 1980s in all three lakes provides further evidence that we have accurately inferred DOC concentrations. Quantitative DOC reconstructions in Sudbury-area lakes offer an excellent technique for assessing past changes in DOC concentrations and UV-B penetration in Canadian Shield lakes.

Résumé : Les approches paléolimnologiques ont fourni de solides modèles d'inférence pour étudier l'acidité et les concentrations de métaux dans les lacs de la région de Sudbury; cependant, on n'a pas encore exploré les données de calibration nécessaires pour pouvoir inférer le carbone organique dissous (DOC) dans l'eau des lacs. Une compilation des données limnologiques existantes a fourni des valeurs de DOC pour 80 des 105 lacs utilisés dans nos calibrations, ce qui nous a permis d'examiner l'importance relative du DOC dans la distribution des communautés de diatomées des lacs du Bouclier canadien, tels ceux de la région de Sudbury. Une analyse des correspondances canoniques (CCA) révèle que la concentration de DOC dans l'eau explique une partie significative de la variation dans les données sur les diatomées. Notre modèle d'inférence du DOC avec moyennes pondérées montre une forte corrélation (r^2 = 0,63) entre le DOC mesuré et le DOC inféré. À l'aide de ce modèle, il a été possible de faire une reconstitution dans le temps du DOC dans trois lacs de Sudbury et ensuite d'estimer les changements temporels de la pénétration de l'ultraviolet-B (UV-B). Ces reconstitutions indiquent que les concentrations du DOC et la pénétration des UV-B dans l'eau ont changé de façon notoire dans deux des trois lacs soumis à la modélisation. L'étroite correspondance entre les valeurs de DOC mesurées et inférées pour les années 1980 dans les trois lacs étudiés démontre la précision de notre inférence des concentrations de DOC. Les reconstitutions quantitatives de DOC dans les lacs de Sudbury sont une excellente technique pour évaluer les changements passés dans les concentrations de DOC et la pénétration des UV-B dans les lacs du Bouclier canadien.

[Traduit par la Rédaction]

Introduction

Acid deposition, climate warming, and increased exposure to UV-B radiation are considered to be major global human stressors to natural ecosystems (Schindler 1998). Besides their individual effects, these stressors can have cumulative and often synergistic effects on aquatic ecosystems (Yan et al. 1996). Lake-water transparency is related to all three of these environmental stressors and is one of the most important variables affecting aquatic ecosystems (Gunn et al. 2001).

Water clarity in oligotrophic lakes is primarily controlled by dissolved organic carbon (DOC). DOC is important in aquatic ecosystems because it is a source of energy for microbial metabolism, influences the penetration of UV-B and

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photosynthetically active radiation, affects the thermal structure of lakes, complexes many metals and nutrients, and affects pH and alkalinity (Williamson et al. 1999). Although lake water DOC concentrations can be influenced by many factors (Hessen and Tranvik 1998), they are often linked to changes in local vegetation. The major allochthonous source is humus from trees (Engstrom 1987) and, especially, coniferous trees, such as spruce. Other major sources include drainage from wetlands (Devito et al. 1989).

Acidification decreases DOC in lakes, either by precipitation with metals (e.g., Al) or by dissociation with H^+. Increased drought frequency can also reduce the export of DOC from the drainage basin and increase lake-water clarity. At the Experimental Lakes Area in western Ontario, DOC concentrations have declined an average of 15–20%, resulting in 22–63% increases in the depth of 1% UV-B penetration in these lakes (Schindler et al. 1996). These changes were attributed to warmer temperatures and lower precipitation in the 1970s and 1980s. Yan et al. (1996) showed that large declines in pH and DOC, and resulting increases in UV-B penetration, were attributable to reoxidation of reduced sulfur compounds from the exposed littoral zone and watershed of a lake after a drought.

Unfortunately, past-measured DOC data are only available for a small number of lakes, and the records often only cover the last ~15–20 years. These data records are too brief to characterize background (i.e., natural) DOC concentrations or to assess past changes in DOC. Long-term water-quality data are essential for the complex task of natural-resource management and to identify the timing, magnitude, and rate of change in surface waters (Smol 1995). In the absence of long-term water-quality data, paleolimnological techniques are being used increasingly to obtain these missing data sets (Stoermer and Smol 1999).

Diatoms are biological indicators widely used to assess past lake water characteristics (S.S. Dixit et al. 1992). Diatoms are single-celled algae that possess a siliceous cell wall or frustule that preserves very well in most lake sediments. The size, shape, and sculpturing of diatom valves are species-specific. Because a large number of diatom species have defined optima and tolerances to many environmental variables, they have been widely used to assess a wide variety of environmental perturbations (Stoermer and Smol 1999).

During the lake-acidification research in Adirondack Park (New York), a statistically significant weighted averaging DOC model was developed, using the diatom assemblages from 71 lakes (S.S. Dixit et al. 1993). Since then, a number of studies have utilized diatom assemblages for DOC or total organic carbon (TOC) reconstructions and to assess past climatic and vegetational shifts in northern ecosystems (e.g., Pienitz and Smol 1993; Pienitz et al. 1999; Rosen et al. 2000). For example, lake water DOC concentrations declined greatly as one crossed the forest–tundra boundary in northwestern Canada (Pienitz et al. 1997). The DOC reconstructions covering the last 7000 years in two Arctic lakes provided evidence that marked limnological changes had occurred and that they were most likely associated with the shift in treeline, which was due to changes in climate (Pienitz et al. 1999). Using diatom-inferred DOC data, Pienitz and Vincent (2000) estimated past underwater light penetration and suggested that lakes located across vegetational gradients would experience significant shifts in underwater light penetration as a result of the effects of climate change on catchment vegetation and the export of DOC.

A large area of the Sudbury (Ontario, Canada) region contains bedrock that is highly resistant to erosion and provides very little buffering. The lakes of this region were among the first sites in Canada to be identified as being affected by acid rain, and there is growing evidence that adverse ecological effects of increasing UV-B penetration are likely significant as well (Gunn et al. 2001).

Extensive paleolimnological studies have been carried out in the Sudbury region, to assess the impacts of industrial activities on lakes, including acidification and metal contamination (S.S. Dixit et al. 1987; A.S. Dixit et al. 1992*a*, 1992*b*). Recently, we have expanded our Sudbury calibration data set, and the available diatom-inference models for lake-water acidity have been further refined (S.S. Dixit et al. 2001). However, DOC had never been included as one of the limnological variables in our calibration studies (S.S. Dixit et al. 2001), as DOC measurements were not available when the original 72 calibration lakes were sampled between 1984 and 1987 (S.S. Dixit et al. 1991).

The Ontario Ministry of Environment (MOE) began monitoring a large number of lakes within 260 km of Sudbury in the 1970s (Keller and Pitblado 1986). Since then, monitoring has continued, albeit at different intensities, to provide regional estimates of acid-affected lakes in the region and to monitor any recovery due to post-1970 reductions in SO_2 emissions. During the 1980s, MOE began to include lake water DOC measurements for selected lakes and, in fact, DOC values are now available for 47 of our original 72 lakes. For the majority (90%) of these 47 lakes, DOC measurements were made at the same time of year (June–August) that sediment cores were collected. Combining these 47 lakes with the 33 new lakes that we have recently added to our expanded calibration data set (S.S. Dixit et al. 2001), gives a total of 80 calibration lakes with DOC data. Lake water DOC data for 36 of the original 47 lakes were collected within 3 years of sediment-core collection, whereas for the remaining 11 lakes, DOC data were collected 6–9 years after sediment-core samples were collected. Although it would be ideal to have DOC data for the time of sediment sampling, these data are not available. The strength of our inference equation, as discussed below, lies in our contention that these DOC data are in fact representative of ambient conditions. The diatom assemblages of these lakes can be studied against a diverse set of environmental variables, including DOC. The spatial distribution of these 80 lakes (Fig. 1) covers the entire area occupied by our 105 calibration lakes (S.S. Dixit et al. 2001).

The objectives of this study were to (*i*) examine the influence of lake water DOC concentrations on surface-sediment diatom assemblages from the 80 Sudbury-area lakes; (*ii*) develop a weighted-averaging diatom-inferred DOC model for this region; (*iii*) apply this model to the reconstruction of past lake water DOC concentrations in three Sudbury-area lakes; and (*iv*) estimate past UV-B penetration in our study lakes, using the inferred-DOC data. In this paper, we mainly present our DOC-related results, whereas details regarding other environmental variables (i.e., pH and metals) are presented elsewhere (S.S. Dixit et al. 2001).

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Materials and methods

The details of our field and laboratory methods are presented in S.S. Dixit et al. (1991, 2001). In summary, sediment cores were collected using a modified K-B gravity corer, and the cores were extruded using a vertical extruding device. The surface-sediment samples were digested in strong acids, and the cleaned slurry was then dried onto cover slips. The cover slips were mounted on glass slides using Hyrax® or Naphrax® mounting medium. A minimum of 500 diatom valves were identified and counted in transects for each surface sample at ×1250 magnification under oil immersion. Identifications were made to the lowest possible taxonomic level, using standard taxonomic literature.

Using the CANOCO (version 4) program (ter Braak and Šmilauer 1998), canonical correspondence analysis (CCA) was performed, to determine which of the environmental variables exerted significant influences on the distributions of the surface-sediment diatom assemblages of our 80 study lakes. CCA is a multivariate direct gradient analysis, where the distribution of species data can be directly related to available environmental data (Birks 1998). In CCA, we used the forward-selection option and Monte Carlo permutation tests, to identify significant environmental variables in our 80-lake data set. We specifically evaluated the significance of DOC for developing an inference model by running constrained and partial CCAs and, then, examined the significance of these CCAs, using Monte Carlo permutation tests on forward-selected environmental variables. In CCA, except for pH and elevation, all environmental variables (conductivity, Cu, Ni, Zn, Al, Fe, Ca, Na, Cl, SO_4, DOC, color, sampling depth, and Secchi depth) were $\log_e (x + 1)$ transformed, to normalize the data. The limnological characteristics of the study lakes are summarized in Table 1. Water-chemistry methods followed MOE (1981). We considered all 248 diatom taxa that were identified in the surface sediments of our 80 lakes, and their relative abundances were $\log_e (x + 1)$ transformed to reduce the influence of dominant taxa in CCA.

The WACALIB (version 3.3) program (Line et al. 1994) was used for developing a diatom-based weighted-averaging (WA) model for inferring lake-water DOC. WA-regression and calibration models are widely used in paleolimnological studies for quantitative reconstructions of past environmental conditions (Birks 1998), including those in the Sudbury region (S.S. Dixit et al. 2001). In WA, a unimodal relationship between species composition and an environmental variable is assumed. The WA DOC inference model was developed using $\log_e(x + 1)$ measured DOC values, $\log_e(x + 1)$ transformed diatom-species data, and the classical deshrinking option in WACALIB. To assess realistic error estimates in inferences, the bootstrap error estimation approach, available in WACALIB, was applied.

Results and discussion

Among the 16 environmental variables considered in the CCA (Table 1), 9 variables (pH, Ni, DOC, Secchi depth, elevation, Ca, Al, Cu, and depth) were identified as significant ($p \leq 0.005$) and collectively explained 76% of the total variance (19%) in the diatom-species data. The variance explained here is almost identical to the variance (77%) explained in our 105-lake calibration data set (S.S. Dixit et al. 2001). Lake-water pH (25%), Ni (10%), and DOC (9%) explained more than half (57%) the explainable variance, with the remaining variance (43%) being accounted for by the remaining six environmental variables.

In a constrained CCA, the ratio of the first constrained eigenvalue (λ_1) to the second unconstrained eigenvalue (λ_2) was 0.56, and the DOC variable was significant ($p \leq 0.005$). In a constrained CCA, the ratio λ_1/λ_2 indicates the relative importance of the environmental variable in explaining the species data. The λ_1/λ_2 for the partial CCA was also high (0.46) and significant ($p \leq 0.005$). Among the forward-selected variables, only pH had a higher λ_1/λ_2 value (0.72) than DOC. Constrained and partial CCA results indicate that a significant proportion of the additional variance in the diatom-species data can be explained by DOC. Because DOC is an important variable controlling the distribution of diatom assemblages, a model for inferring lake-water DOC in Sudbury-area lakes can, potentially, be developed.

Using the diatom assemblages for all 80 Sudbury-area lakes, we developed a WA model to infer lake-water DOC (Fig. 2*a*). The correlation between measured and inferred DOC was strong ($r^2 = 0.63$; bootstrap root mean square error ($RMSE_{boot}$) = 0.38 mg·L^{-1}), and the residuals were small and evenly distributed (Fig. 2*b*). We also examined the influence of the 11 lakes for which measured DOC data were collected 6–9 years after sediment-core collection. This was done by excluding these lakes from the 80-lake data set and developing a 69-lake WA model for DOC (Fig. 2*c*). The statistics for the 69-lake model ($r^2 = 0.61$; $RMSE_{boot}$ = 0.40 mg·L^{-1}) were similar to those for the model based on all 80 lakes; the residuals (Fig. 2*d*) also followed a similar distribution pattern in the two models. These data suggest that including the 11 lakes did not have an adverse effect on our DOC model. This is likely the case, because the DOC levels in these 11 lakes did not change much within the last 6–9 years, most probably because DOC concentrations in these 11 lakes were moderate to high and none of the lakes were very acidic (DOC 1.9–7.1 mg·L^{-1}; pH 6.2–7.6). Snucins and Gunn (2000) indicated that lakes with high DOC are less susceptible to declines in DOC due to climate change and (or) acidification than clear-water acidic lakes.

Although including these 11 lakes did not improve our DOC model, adding these lakes to our 80-lake calibration data set would provide better estimates of DOC optima for diatom taxa found in Sudbury-area lakes. Among the 248 diatom taxa identified in this study, 142 were present in at least 10 lakes and were considered common.

The observed relationship between measured and inferred DOC compares closely with the DOC models developed for lakes in the Adirondacks, New York ($r^2 = 0.64$) (S.S. Dixit et al. 1993), and in the Northwest Territories, Canada (r^2 = 0.66) (Pienitz and Smol 1993). These comparisons suggest that our diatom-based DOC model is fairly robust and can provide useful information on past DOC concentrations in Sudbury-area lakes.

The applicability of our DOC model was further evaluated by reconstructing the past DOC concentrations in Clearwater, George, and Whitepine lakes, located within our study region (Fig. 1). Clearwater Lake is located 12 km south of Sudbury, whereas Whitepine Lake is located 90 km north of Sudbury. George Lake is located about 60 km southwest of Sudbury, in Killarney Provincial Park. The detailed diatom profiles and lake water pH reconstructions for these lakes are already published (S.S. Dixit et al. 1987; A.S. Dixit et al. 1992*a*, 1993). The past-DOC inference data (Fig. 3*a*) were computed using the DOC model developed in this study, whereas the pH-inference data (Fig. 3*b*) are from earlier publications.

In addition to our inferred data, measured DOC and pH data were also available for the study lakes, beginning in the

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Fig. 1. Study area and location of the 80 calibration lakes selected for developing the diatom-based dissolved organic carbon (DOC) inference model for Sudbury-area lakes. The lakes (Clearwater, George, and Whitepine) selected for the downcore DOC reconstructions are indicated by "★."

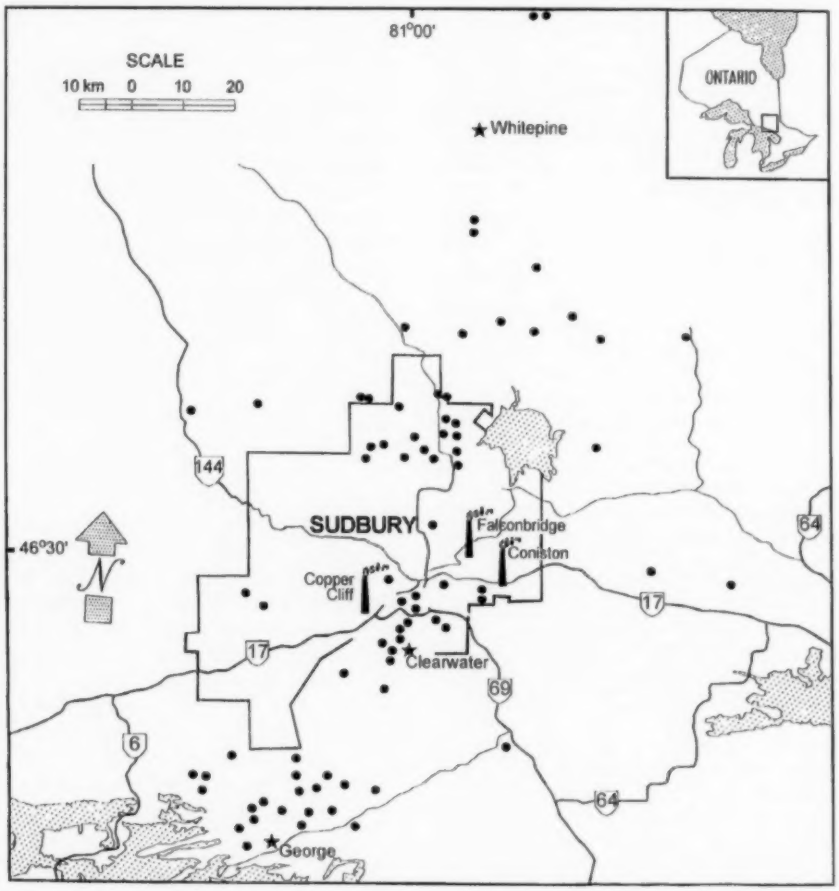

Table 1. Minimum, maximum, and mean values of selected limnological characteristics for the 80 calibration lakes.

Variables	Minimum	Maximum	Mean
Elevation (m)	182.0	430.0	286.6
Sampling depth (m)	4.0	39.0	17.9
Secchi depth (m)	1.6	30.9	7.0
pH	4.2	7.7	6.1
Conductivity ($\mu S \cdot cm^{-1}$)	21.0	367.0	63.6
Calcium ($mg \cdot L^{-1}$)	1.0	32.1	4.6
Sodium ($mg \cdot L^{-1}$)	0.4	34.8	3.5
Chloride ($mg \cdot L^{-1}$)	0.2	53.5	3.9
Sulphate ($mg \cdot L^{-1}$)	5.0	137.0	13.8
Dissolved organic carbon ($mg \cdot L^{-1}$)	0.1	11.3	2.6
Color (true color)	0.0	60.0	7.1
Total aluminum ($\mu g \cdot L^{-1}$)	7.0	950.0	105.8
Total copper ($\mu g \cdot L^{-1}$)	0.0	366.0	16.8
Total nickel ($\mu g \cdot L^{-1}$)	0.4	2100.0	78.8
Total zinc ($\mu g \cdot L^{-1}$)	0.0	800.0	31.2
Total iron ($\mu g \cdot L^{-1}$)	0.0	520.0	88.7

early 1980s. These data are plotted in Fig. 3. Including measured data allowed us to expand the recent temporal record of trends in DOC. It also allowed us to compare the measured and inferred data that were available from the 1980s. The inferred DOC data for the 1980s closely match (r^2 = 0.91; standard error = 0.29 $mg \cdot L^{-1}$) the available measured DOC data for the same period in all three lakes (Fig. 3*a*). These data further suggest that past lake water DOC concentrations in Sudbury-area lakes can be accurately inferred using diatom assemblages.

Our paleolimnological data indicate that DOC concentrations have declined in the past in all three study lakes (Fig. 3*a*). DOC concentrations were historically low in Clearwater and George lakes, whereas levels were comparatively higher in Whitepine Lake. In Clearwater Lake, the pre-1910 inferred DOC concentrations were 1.5–1.8 $mg \cdot L^{-1}$. However, the concentrations have declined since then, reaching their lowest value of about 0.5 $mg \cdot L^{-1}$ in the 1960s and remaining low until about 1980. Lake-water pH had also declined in Clearwater Lake (Fig. 3*b*). The inferred pH (5.8–6.1) remained relatively unchanged until about 1910, but then declined to a low of ~4.7 in the early 1980s. The available measured DOC and pH data indicate that lake-water

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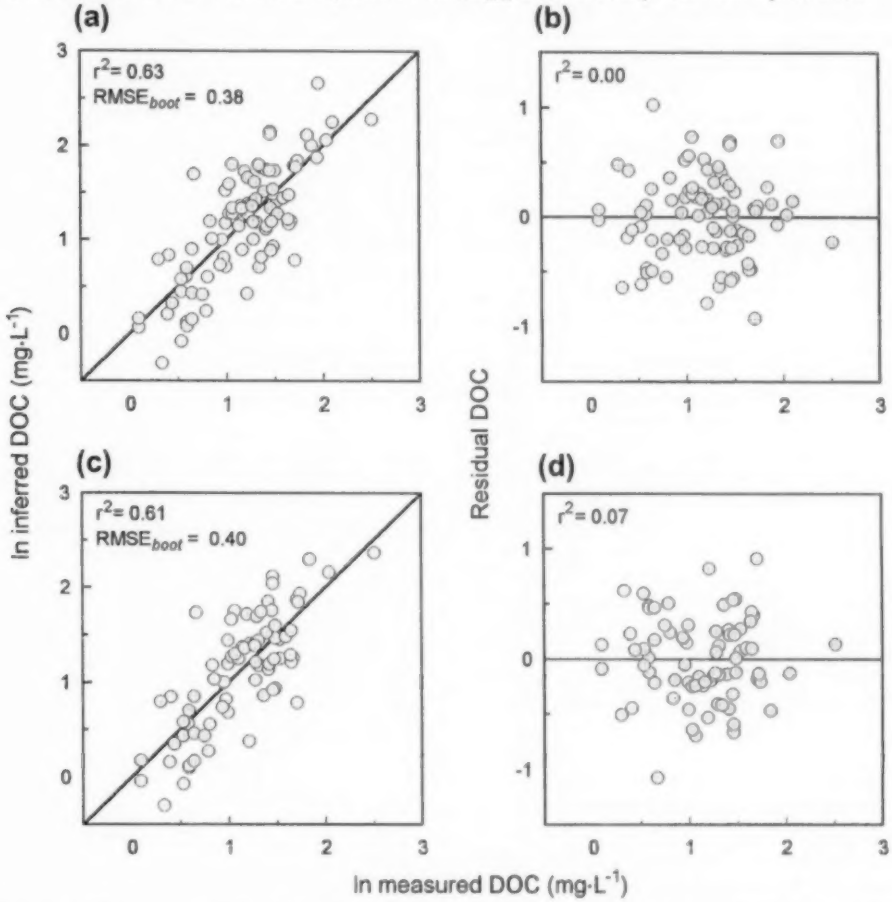
Fig. 2. The relationship between $\log_e$ measured dissolved organic carbon (DOC) and diatom-inferred DOC (*a*) and residuals (relationship between measured versus measured minus inferred DOC) (*b*) for the 80-lake calibration data set. The calibration relationship for the 79-lake data set (*c*) and their residuals (*d*) are also presented. $RMSE_{boot}$ is the bootstrap root mean square error.



DOC and pH have rapidly recovered in Clearwater Lake within the last 20 years (Fig. 3). Recent (i.e., 1999) measured DOC (2.2 $mg \cdot L^{-1}$) and pH (6.0) values are close to the values (DOC 1.6 $mg \cdot L^{-1}$; pH 5.9) inferred from the diatom assemblages preserved in sediments for the ~1840s.

In George Lake, pre-1920 inferred DOC concentrations ranged between 1.7 and 2.5 $mg \cdot L^{-1}$ (Fig. 3*a*). However, since then, DOC levels have declined, reaching their lowest value of 1.2 $mg \cdot L^{-1}$ in ~1970. Over this period, inferred pH also declined, from a high of about 6 before 1920 to a low of 5.4 in ~1970 (Fig. 3*b*). As in Clearwater Lake, lake-water DOC and pH in George Lake have increased rapidly since the 1980s, and values measured recently closely match the pre-1920 inferred values. These data strongly suggest that, despite the past declines in DOC and pH, lake-water DOC and pH have now recovered to pre-industrial levels in both Clearwater and George lakes.

In Whitepine Lake, inferred DOC concentrations increased slightly (2.9–3.5 $mg \cdot L^{-1}$) between ~1850 and ~1920 but, by the early 1980s, the concentrations had declined from 3.5 to 2.2 $mg \cdot L^{-1}$ (Fig. 3*a*). The inferred pH profile indicates that the pre-1920 pH ranged between 6.4 and 6.6, and that pH started to decline distinctly about 1920. In fact, lake-water pH declined nearly 0.8 of a pH unit (from 6.6 to 5.8) between ~1920 and ~1970, with a sharp drop in pH between ~1960 and ~1970 (Fig. 3*b*). During the 1970s and early 1980s, the inferred pH (5.8–6.0) remained relatively unchanged. Although measured pH data for Whitepine Lake indicate that lake-water pH has recently recovered to a high of 6.3 (Fig. 3*b*), the measured DOC levels have only slightly increased over the last ~20 years, unlike Clearwater and George lakes.

The penetration of UV-B in Canadian Shield lakes is largely a function of the concentration of DOC, and empirical models have previously been developed for computing the depth of UV-B penetration (Scully and Lean 1994; Schindler et al. 1996; Yan et al. 1996). We estimated past UV-B penetration in our study lakes (Fig. 3*c*), using both inferred- and measured-DOC data. The 1% UV-B depth was calculated from the diffuse vertical attenuation coefficient (K*d*), using an equation ($Kd = 0.60 + 0.389DOC^{1.873}$; $r^2 = 0.97$) used earlier for Sudbury-region data by Yan et al. (1996). Although the relationship between DOC and UV attenuation is quite good, it is important to point out that the equation does not take into account differences in the optical properties of different types of DOC. DOC comprises a very

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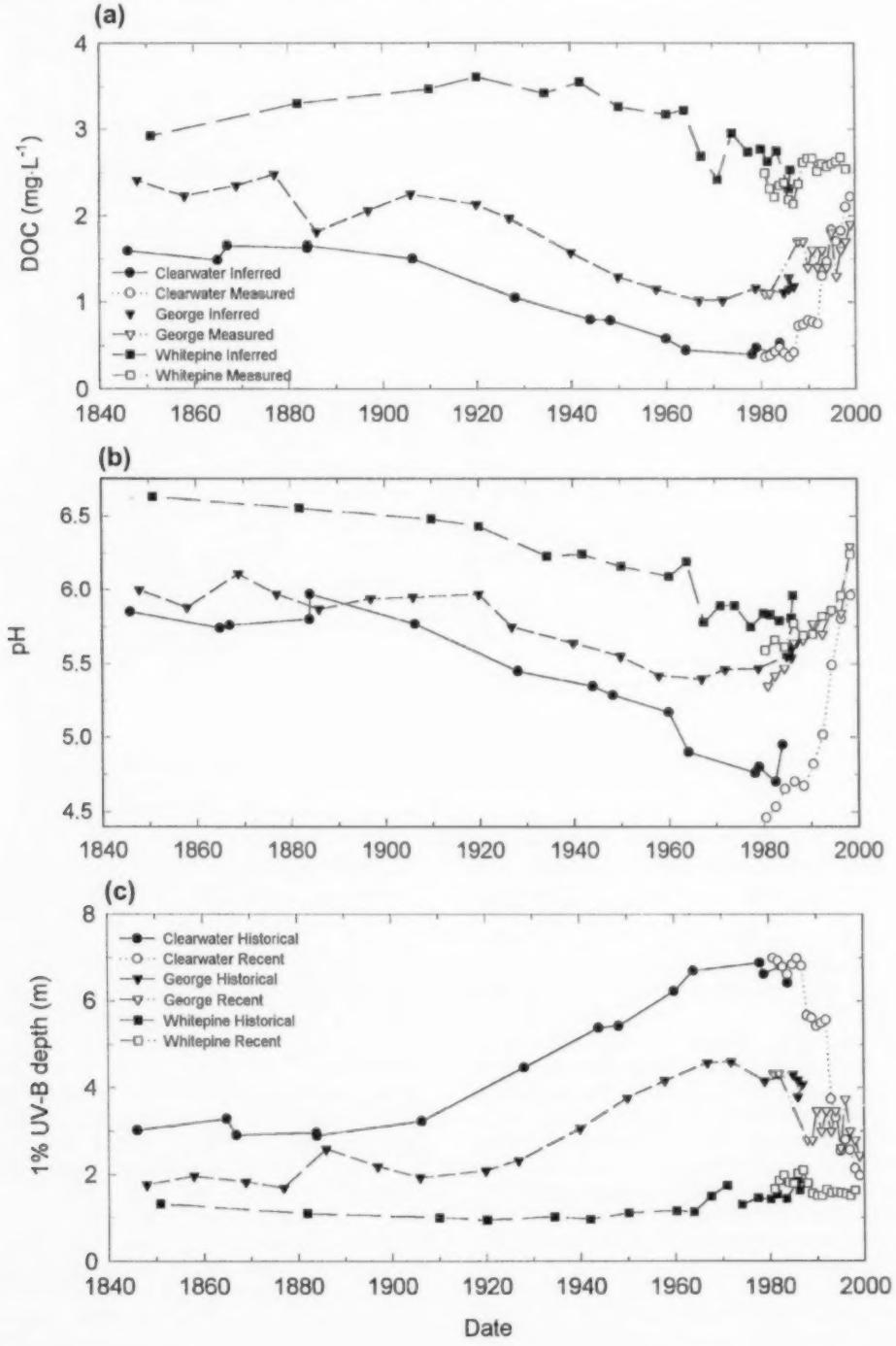
Fig. 3. Downcore dissolved organic carbon (DOC) (*a*), pH (*b*), and 1% UV-B penetration depth (*c*) for Clearwater, George, and Whitepine lakes. Measured DOC and pH values that have been available since 1980 are also shown. To avoid the cluttering of measured pH data points, we have presented 2-year lake water pH averages. Both measured and inferred DOC data were used in estimating 1% UV-B penetration depth for historical and recent times. The same symbols are used in both *a* and *b*.



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broad class of chemically and optically diverse compounds that may vary among lakes (Williamson et al. 1996). Moreover, although differences in particulate matter (e.g., plankton, detritus) were not important in explaining the variability in light attenuation among these lakes, they may be important within a lake.

The inferred UV-B data indicate that, as Clearwater Lake acidified, the 1% UV-B penetration depth increased from about 3 m in ~1910 to 7 m in the late 1970s (Fig. 3*c*). However, corresponding to the recent increase in lake-water pH, the depth of water exposed to 1% of lake surface UV-B irradiance has now returned to "background" (i.e., pre-1850) levels. In George Lake, the 1% UV-B penetration depth increased modestly (from ~2 m to 4.5 m) between ~1920 and 1970 and, since then, has returned to background levels. In Whitepine Lake, changes in UV-B penetration have been relatively small over the last ~150 years; the 1% UV-B penetration depth changed from a background value of ~1–2 m during maximum acidification and then recovered to background levels.

Schindler et al. (1996) indicated that, in boreal lakes, DOC concentrations of several milligrams per litre may restrict penetration of UV-B to only the upper layers of water and that these levels are sufficient to provide an effective shield against ultraviolet radiation for aquatic organisms. Although DOC concentrations in Whitepine Lake have largely provided this protection throughout the last ~150 years (covered by our study), the protective shield was gradually lost in Clearwater and George lakes as these lakes acidified. In Clearwater Lake, UV-B penetration increased exponentially as inferred DOC declined. While the magnitude of change was different in Clearwater and George lakes, the patterns of change were about the same.

Synthesis and conclusions

The decline in DOC and the resulting increase in UV-B penetration over the last century in our study lakes, and their recent recoveries, are closely associated with changes in lake-water pH (i.e., acidification and recovery). However, the interpretation of DOC trends over the last century or so may be somewhat complicated, owing to interactions with multiple stressors (e.g., acid rain, climate change, vegetational shifts). For example, inferred pH declined in Clearwater Lake from a pre-1910 background value of about 5.7–6.0 to a value of 4.7 in ~1980. The initial lake water DOC changes in our study lakes may have commenced somewhat earlier than the metallurgical activities in the Sudbury basin and before our oldest inferences. Although both climate warming and acidification may be responsible for declines in DOC and resulting increases in light penetration, even longer-term DOC data are necessary to assess the relative importance of recent acidification and the climate-related changes to lake-water DOC. The possible effects of other historical watershed disturbances, such as logging and fires, may also have been significant. To understand the relative importance of these factors, we plan to initiate further long-term studies on selected Sudbury-area lakes.

In small oligotrophic Canadian Shield lakes, lake water DOC concentrations have significant effects on light penetration, and DOC is the most important predictor of thermocline depth (Pérez-Fuenetaja et al. 1999). Lake water DOC concentrations play a critical role in underwater light penetration and resulting UV-B radiation. UV-B penetration depth is relatively stable over a DOC range of 4–11 $mg \cdot L^{-1}$ but increases markedly with decreasing DOC; thus, DOC acts as a "natural sunscreen" (Vincent and Pienitz 1996).

Sudbury-area lakes are likely to be sensitive to increased UV-B radiation, because many poorly buffered lakes in the region contain relatively low DOC. Our inference data suggest that, as DOC concentrations declined, UV-B penetration increased substantially in two of our study lakes, with the maximum increase occurring in the lake (Clearwater) that experienced the most severe acidification in the past. UV-B penetration likely removed some of the DOC in the surface layers and, also, likely caused changes in the absorbance characteristics of the DOC (Moran and Zepp 1997; Morris and Hargreaves 1997). In turn, these changes would have caused light (including UV-B) to penetrate deeper in the water column, causing further photodegradation and changes in thermal structure.

Our past paleolimnological research in the Sudbury region has shown that metal mining and associated smelting activities have resulted in marked acidification in many of the poorly buffered lakes over the last ~100 years. Recent lake water pH recovery has also occurred in some lakes, owing to reductions in sulphur emissions in the region since the 1970s (Keller et al. 1999). With the availability of this diatom-inferred DOC model, we are now able to estimate past lake-water concentrations of DOC and provide long-term data concerning the onset, rate, and magnitude of UV-B penetration changes in Canadian Shield lakes. Our data will also be potentially useful in paleoclimatic interpretations and for correlating and evaluating data generated from other biological indicators (e.g., chironomids and pollen) and geochemical markers.

The effects of increased UV-B penetration on biota in Precambrian Shield lakes are not well known but, potentially, may be severe. Long-term data from paleoecological studies should help lake managers and scientists to understand the potential influences of changing light penetration and related changes in thermal structure on both aquatic-plant and -animal communities. Such records are fundamental for a better understanding of the potential future implications of climate change.

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The importance of influence diagnostics: examples from Snake River chinook salmon spawner–recruit models

Richard A. Hinrichsen

Abstract: The rapid decline of some salmonid populations in the Columbia River Basin led investigators to analyze spawner–recruit (SR) data in order to understand the potential gains of improving main-stem passage conditions and quantify the effectiveness of the juvenile transportation program. Direct measurements of passage survival and transportation were not always available, so instead, the researchers attempted to tease out the passage or transportation effects by using trends in production estimated from SR models. Small subsets of data, or even single observations, highly influenced the estimates of passage survival and transportation effectiveness derived from these models. For stream-type chinook salmon, deleting 1 of 13 stocks changed the estimate of main-stem passage survival from 11 to 34%. For ocean-type chinook salmon, the conclusion that transportation should be immediately halted hinged on a single observation. The Snake River salmon SR models starkly illustrate the importance of using influence diagnostics to temper inferences.

Résumé : Le déclin rapide de certaines populations de salmonidés dans le bassin du fleuve Columbia a amené des chercheurs à analyser les données sur les reproducteurs et le recrutement (données SR) pour évaluer les gains potentiels qu'il y aurait à améliorer les conditions de migration des poissons dans le cours principal et pour quantifier l'efficacité du programme de transport des juvéniles. Comme les mesures directes de la survie durant la migration et les données sur le transport ne sont pas toujours disponibles, les chercheurs tentent d'extraire les effets de la migration ou du transport à partir de tendances dans la production estimées par des modèles SR. Des petits sous-ensembles de données, et même des observations individuelles, ont une grande influence sur les estimations de survie durant la migration et d'efficacité du transport dérivées de ces modèles. Chez le Saumon quinnat de rivière, le retrait d'1 des 13 stocks a fait varier l'estimation de la survie à la migration dans le cours principal de 11 à 34%. Chez le Saumon quinnat de mer, la conclusion d'interrompre le transport reposait sur une seule observation. Les modèles SR utilisés pour les saumons de la rivière Snake illustrent de façon décisive l'importance de déterminer les facteurs qui influencent un modèle afin de moduler les inférences qu'il génère.

[Traduit par la Rédaction]

Introduction

Assessing the opportunities to rehabilitate Snake River chinook salmon (*Oncorhynchus tshawytscha*) runs requires estimating the effects of passage and the transportation program on migrant-smolt survival. Passage mortality and transportation effectiveness were estimated from spawner–recruit (SR) models by the PATH process (Plan for analyzing and testing hypotheses; National Marine Fisheries Service 1995), because treatment–control experiments were lacking. For Snake River stream-type chinook salmon, treatment–control experiments were available for measuring certain reach survivals, but total passage survival estimates were based on extrapolating the reach measurements to the entire migration corridor. For Snake River ocean-type chinook salmon, no experiments have measured the survival of transported juvenile migrants relative to their untransported counterparts. Non-experimental SR data were, therefore, used to draw inferences about passage mortality and the effectiveness of transportation.

The DELTA SR model was developed to empirically estimate passage mortality by comparing spawning and recruitment data for seven Snake River and six lower Columbia River stream-type chinook salmon populations (Deriso et al. 1996). The technique compared trends in Snake stocks (treatment group) and Lower Columbia stocks (reference group). Productivity was defined as the natural logarithm of the ratio of recruits minus the density-dependent mortality. This corresponds to the Ricker-a value in the usual SR Ricker equation: $\ln(R/S) = a - bS$. Passage mortality estimates were derived by assuming that, if not for the Snake River dams, the fluctuations of density-independent Ricker-a values of the Snake and Lower Columbia stocks would be similar over time (allowing for some level of normally distributed process error). This controversial assumption (Zabel and Williams 2000) permitted estimation of the population dynamics of Snake index stocks, had the Snake River dams not been constructed, which, in turn, allowed estimation of passage

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mortality. The stream-type chinook salmon SR data set was itself derived from many assumptions, but it is the best available (Beamesderfer et al. 1997).

The FALLD SR model was developed to empirically estimate the smolt-to-adult survival of transported Snake River ocean-type chinook salmon relative to that of their untransported counterparts (Peters et al. 1999). This quantity is essential to determine whether transportation should be halted or increased in an attempt to rehabilitate the Snake River ocean-type chinook salmon run. The FALLD model used SR data from two different ocean-type chinook salmon stocks: those that spawn in the Snake River upstream of Lower Granite Dam (697 km upstream of the ocean) and those that spawn in the Deschutes River (342 km upstream of the ocean). Estimates of yearly passage mortality and fraction of juveniles transported were model inputs. Unfortunately, the SR data for ocean-type chinook salmon in the lower Columbia River were insufficient to estimate passage mortality, so passage model estimates were entered directly into the SR model (Peters et al. 1999).

Because of the layers of modeling and data derivation, I was concerned about the sensitivity of the substantive inferences to small data perturbations. This concern is often not addressed by fisheries scientists, perhaps owing to the mistaken notion that all observations in a regression analysis have equal influence on the parameter estimates. Least squares (LS) estimation can be highly sensitive to individual observations. For example, in a LS analysis of smolt survival versus migration survival of Columbia River stream-type chinook salmon, 2 low years, 1973 and 1977, largely defined the apparent relationship (Sims and Ossiander 1981). This is a common difficulty with non-experimental data, which often do not provide good coverage of the variate space and are subject to wide variation in response. Frequently, when using non-experimental data, the apparent explanatory power of a covariate is associated with just a few observations, in which case there is a high probability that the apparent relationship between the response and covariate is not causal (Hilborn and Walters 1992). To detect such situations, influence diagnostics are used (Belsley et al. 1980; Cook and Weisberg 1982). Most diagnostics involve systematically removing a subset of the data, re-estimating the model parameters, and then comparing these new estimates with the original estimates.

My main objective was to expose single observations and groups of observations that were highly influential on the inferences drawn on passage mortality, transportation effectiveness, and other important quantities used in the PATH SR models. Secondly, I sought to explain the exceptional influence of certain observations by scrutinizing the data sets and the model structures. My hope is that these illustrations, drawn from models used to inform decisions on breaching Snake River dams, will prompt fisheries researchers to routinely use influence diagnostics in the analyses of fisheries data. These diagnostics can be every bit as relevant as confidence intervals and standard errors when drawing inferences from non-experimental data.

Materials and methods

The data employed are Columbia River stream-type chinook salmon SR data (Beamesderfer et al. 1997) and Snake River ocean-type chinook salmon SR data (Peters et al. 1999). Analysis of these SR data employs Ricker models modified to include life-stage survival factors. The stream-type chinook salmon model, DELTA, uses only SR data as input, while the ocean-type chinook salmon model, FALLD, uses SR data, estimates of passage mortality, and fraction of fish transported.

The Snake River stream-type chinook salmon model (DELTA)

The DELTA model was derived for the analysis of the Snake River stream-type chinook salmon SR data of brood years 1952–1990 (Deriso et al. 1996). The data include 13 index stocks: seven threatened stocks from the Snake River (treatment stocks) and six stocks from the lower Columbia River (reference stocks) (Schaller et al. 1999). The model DELTA uses parameters that vary yearly to model changes in the density-independent productivity and survival processes:

$$(1)\quad \ln(R_{t,i}) = \ln(S_{t,i}) + a_i - b_i S_{t,i} - \mu_t - n_{t,i}X + \delta_t + \varepsilon_{t,i}$$

where $R_{t,i}$ is the estimated Columbia River returns (recruitment) originating from spawning in year t and stock i; $S_{t,i}$ is the observed spawners; a_i is the density-independent Ricker-a parameter for stock i; b_i is the Ricker-b parameter for stock i; μ_t is the passage mortality due to the Snake and McNary dams after 1969 ($\mu_t = 0$ for $t < 1970$); $n_{t,i}$ is the number of dams through which a stock must pass in year t (if prior to 1970) and the number of dams downstream of McNary Dam (river km 470 of the Columbia River) through which a stock must pass in year t (if after 1969); X is the dam passage mortality per dam; δ_t is the common-year effect (common to all stocks); and $\varepsilon_{t,i}$ is a normally distributed error term.

The model parameters consist of 38 δ_t, 21 μ_t, 13 a_i, 13 b_i, and one dam mortality parameter, X, yielding a total of 86. This required estimation of only 37 δ_t parameters, since the 1990 δ is minus the sum of the first 37 (the complete series is constrained to sum to zero, because it represents a sequence of anomalies from the mean). The total number of observations was 406. Further details of the model and its rationale are provided in Deriso et al. (1996).

To simplify, I calculated the influence of observations of Snake River stocks on four summary parameters instead of the full 86. I fit the full 86-parameter model, but examined only effects of data perturbations on these four summary parameters: (1) the average Snake River Ricker a; (2) the average Snake River Ricker b; (3) the average passage mortality for the Snake River stocks, $\mu_t + n_t X$ (1970–1990); and (4) the average shift in the year effect parameter series δ_t for 1975 (difference between the average δ_t over 1952–1974 and the average over 1975–1990), which is referred to as the regime-shift parameter (this parameter was used to estimate the effect of the ocean–climate regime shift corresponding to brood year 1975 (Beamish et al. 1999)). Summarizing in this way takes a complex (probably overparameterized) model and reduces it to a few useful elements.

The Snake River ocean-type chinook salmon model (FALLD)

The FALLD model was developed to investigate the relative benefit of transportation versus dam breaching for Snake River fall chinook salmon. The model was fit to SR data and passage model output described by Peters et al. (1999). In this model, the chief parameter of interest, D, represents the relative survival of transported smolts versus nontransported smolts from the point of release below Bonneville Dam (river km 235.6) to adulthood. The life-cycle model describing the dynamics of the Snake River ocean-type chinook salmon was (Peters et al. 1999)

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$$(2)\quad \ln(R_t) = \ln(S_t) + a - bS_t + g\cdot\mathrm{dres}_t - M_t + \ln(DP_t + 1 - P_t) + \varepsilon_t$$

$$S_t = S_{\mathrm{wild},t} + E \cdot S_{\mathrm{supp},t}$$

where R_t is recruitment, S_t are spawners, a is the Ricker-a parameter, b is the Ricker-b parameter, g is a coefficient that measures the association between the Snake and Deschutes river recruitment patterns, dres, is a residual from a fit of a two-parameter Ricker model to the Deschutes ocean-type chinook salmon SR data, M_t is an estimate of passage mortality (passage-model derived), D is the smolt-to-adult survival of transported fish (survival from their release downstream of Bonneville Dam to their recovery as adults) relative to the smolt-to-adult survival of their untransported counterparts, P_t is the fraction of fish arriving downstream of the Bonneville Dam that were transported, ε_t is a normally distributed error term, $S_{\mathrm{wild},t}$ is the number of wild spawners, E represents the effectiveness of supplemented spawners, and $S_{\mathrm{supp},t}$ is the number of supplemented spawners. This model is nonlinear in its parameters, since it involves estimating D and E, which are both part of an argument of the natural log function, ln(). The nonlinear LS procedure involved estimating $p = 5$ parameters from a total of $n = 28$ observations (1964–1991).

Influence diagnostics

Cook's distance for an observation, with respect to a given parameter, is defined as: [(change in parameter estimate when observation(s) deleted)/SE]^2, where SE is the standard error of the parameter estimate with all the observations present. This measure is an extension, proposed by Cook (1977), of the usual Cook's distance measure that gauges the change in parameter estimates as a whole when observations are omitted (Weisberg 1985). In this paper, it is how individual parameter estimates change that is of interest, because the important inferences are parameter-specific (e.g., inferences about transportation effectiveness or passage mortality). In practice, observations (or small subsets of observations) that have a Cook's distance greater than one are considered influential. This rough guide, when applied to the single-parameter version of Cook's distance, is equivalent to saying that deletion of an observation moves the parameter estimate by at least 1 SE. Cook's distance and leverage (explained below) are diagnostic statistics only, and are distinguished from other statistics in that no pretense is made to give them inferential content by assigning them distributional properties (Belsley 1991). One does not conclude, for example, that certain values of Cook's distance are significantly different from zero. The diagnostic is used only to alert the investigator to observations that are unusually influential.

I also examined the data sets for observations with high leverage. In a linear model, the leverage of the ith observation is the ith diagonal entry of the hat matrix (Hoaglin and Welsch 1978). Leverage is a straightforward measure of the sensitivity of the fitted line to a perturbation in the observed response: it is the change in predicted response divided by the change in the observed response. (Leverage, defined in this way, assumes that the response is $\ln(R/S)$ not R. If R were used instead, a different set of observations may be flagged as having "high leverage" and leverage would not simply be the ith diagonal entry of the hat matrix.) In a nonlinear model, the leverage of the ith observation is the ith diagonal entry of the tangent plane hat matrix (St. Laurent and Cook 1992, 1993). This version of leverage was necessary for analyzing the nonlinear FALLD model. Like its linear counterpart, it represents the change in an observation's predicted response divided by an infinitesimal change in its observed response.

Generally, an observation has high leverage when it lies far from the bulk of the other observations in the space of explanatory variables. In the nonlinear model FALLD, for example, leverage of an observation is high when one of its explanatory values (e.g., P_t) is far from average (e.g., $\bar{P} = \Sigma_{t=1}^{n} P_t$). The leverage of an observation, h_{ii}, is bounded below by $1/n$ and above by 1 (Weisberg 1985). Hoaglin and Welsch (1978) showed that as h_{ii} approaches its theoretical maximum of 1, the residual for that observation approaches zero. In other words, an observation with high leverage will strongly pull the regression surface toward it, regardless of the value of its response (Belsley 1991); thus, whatever noise is associated with the observation tends to be interpreted as part of a signal. An observation deserves special scrutiny when its leverage exceeds the cutoff value of 2 × (number of parameters)/(number of observations) (Belsley et al. 1980).

It is possible that small groups of observations taken together are influential, even though they are not influential individually. Deletion of individual observations as well as of certain groups of observations (e.g., individual stocks) is straightforward; however, the general problem of identifying all possible groups of observations that are influential presents a combinatorial nightmare when all possible multiple deletions are employed. There are ingenious attempts to circumvent this difficulty (Cook and Weisberg 1982; Gray and Ling 1984), but they are not pursued here.

A careful investigation not only identifies the influential observations; it also tries to understand exactly why the observations are influential (Weisberg 1985). To this end, I examined trends in the SR data, model structures, and correlations among parameters.

Additional model checks

Other model checks that affect the inferences drawn on key parameters were also performed. These included bootstrap bias estimates: a test for normality of errors that uses normal probability plots and a test of autocorrelation of errors that uses a first-order autoregressive procedure.

The bootstrap method proceeded by bootstrapping the residuals (Efron and Tibshirani 1993). In this method, $B = 1000$ bootstrap data sets were generated by resampling (randomly selecting with replacement) the residuals of the LS fit and then adding these to the LS predicted values. Each of these bootstrap data sets was then fit using LS, and the resulting parameter estimates formed a set of 1000 bootstrap replications of the parameter estimates. Bootstrap bias estimates were then calculated for each parameter by subtracting the original LS estimate from the sample mean of the bootstrap replications.

Formal tests for normality of the error terms were conducted by calculating the coefficient of correlation between the residuals and their expected values under normality. High values of the correlation coefficient indicate near normality. The significance level was set at $\alpha = 0.05$; critical values of the test of the null hypothesis of normality were obtained using the methods of Looney and Gulledge (1985).

A test for first-order autocorrelation in the error terms was conducted by jointly fitting a first-order autoregressive (AR) parameter in the original regression models (Quinn and Deriso 1999). The error process was modeled as:

$$(3)\quad \varepsilon_{t+1} = \phi\varepsilon_t + \varepsilon'_t$$

where ε'_t is an independent and identically distributed normal error term and ϕ is the first-order autoregressive parameter. Adding this error process to the models turned them into nonlinear time series models, which were fit using a Gauss–Newton minimization method applied to LS regression (Bates and Watts 1988; Bates and Chambers 1992). The estimate of ϕ was then compared with its standard error to determine significance of the first-order autocorrelation in the error process.

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Table 1. Influence of each stock on the DELTA model summary parameter estimates; here *d*, Cook's distance, represents the squared number of standard errors that a parameter shifts when a stock is deleted (a shift of 1 is generally considered large).

	Passage mortality		Ricker *a*		Ricker *b*		Regime shift	
Stock deleted	Value	*d*	Value	*d*	Value ($\times 10^3$)	*d*	Value	*d*
None	2.238	0.000	3.132	0.000	1.223	0.000	–0.371	0.000
Lower Columbia stocks								
Wind	2.187	0.004	3.090	0.003	1.222	0.000	–0.369	0.000
Klickitat	2.595	0.185	3.451	0.197	1.222	0.000	–0.428	0.068
Warm Springs	2.327	0.011	3.206	0.011	1.223	0.000	–0.275	0.194
John Day Main	2.306	0.007	3.189	0.006	1.227	0.001	–0.379	0.001
John Day Middle Fork	1.093	1.904	2.195	1.694	1.220	0.000	–0.684	2.076
John Day North Fork	2.941	0.717	3.678	0.576	1.224	0.000	–0.085	1.733
Snake stocks								
Imnaha	2.666	0.265	3.513	0.280	1.343	0.581	–0.377	0.001
Minam	2.354	0.019	3.160	0.002	1.106	0.539	–0.307	0.088
Bear Valley	2.098	0.029	3.001	0.033	1.309	0.295	–0.391	0.009
Marsh Creek	2.102	0.027	3.039	0.017	1.195	0.032	–0.395	0.012
Sulphur Creek	1.992	0.088	2.872	0.130	1.002	1.941	–0.411	0.034
Poverty Flats	2.167	0.007	3.111	0.001	1.465	2.331	–0.392	0.009
Johnson Creek	2.251	0.000	3.169	0.003	1.128	0.356	–0.380	0.002

Results

Snake River stream-type chinook salmon

For the stream-type chinook salmon, the 13 index stocks were deleted one at a time to investigate each stock's influence on the four summary parameter estimates (Table 1). Removing a single Lower Columbia (reference) stock, the John Day Middle Fork stock (JDMF), had three large effects: (1) it lowered the estimate of passage mortality from 2.24 (SE = 0.83) to 1.09, tripling the estimate of the passage survival, which is defined as exp(–passage mortality); (2) it decreased the average intrinsic productivity estimate of the Snake stocks from 3.13 (SE = 0.72) to 2.19, decreasing the estimate of pre-dam recruits per spawner at low spawner numbers from 22.9 to 8.9; and (3) it decreased the regime shift estimate from –0.37 (SE = 0.22) to –0.68, representing a change from a statistically nonsignificant 30% drop in recruits per spawner to a significant 50% drop. Deleting each case in turn (from all cases in the data set) revealed that there were few observations (1959, 1964, and 1968) that drove the JDMF stock's high influence (Fig. 1). Though these three observations were highly influential as a group, none had a Cook's distance greater than 1 or leverage greater than half the usual cutoff of 2 × (number of parameters)/(number of observations) = 0.42. Nor were their Studentized residuals (the residuals divided by their standard deviations) extraordinary: –2.69, –2.87, and 1.99 for the 1959, 1964, and 1968 observations, respectively. The reason for their high joint influence was subtle and will be revealed in Discussion.

I diagnosed the reason for the high influence of the 1959, 1964, and 1968 observations by examining trends in the SR data. The observations were influential because they portrayed the Lower Columbia stocks as having a large increase in productivity from 1959 to 1968 that was unmatched by the Snake stocks. The DELTA model interpreted this unmatched increase in productivity as an effect of the installation of two dams, Lower Monumental (river km 67.1) and Little Goose (river km 113.4), completed in 1969 and 1970, respectively, which first affected outmigrants from brood years 1967 and 1968, respectively. According to the data available, the JDMF was the only Lower Columbia stock that showed a sizeable increase in productivity during 1959–1968 (Fig. 2). The JDMF $\ln(R/S)$ rose from an average of 1.21 during 1959–1966 to 2.84 during 1967–1968. The $\ln(R/S)$ for the Snake River stocks rose only slightly, from 0.98 to 1.14, during the same time period. The increase in productivity for the JDMF was unusual, because for the other Lower Columbia stocks, the average $\ln(R/S)$ decreased from an average of 1.59 (years 1959–1966) to 1.49 (years 1967–1968). Overall, the reason for the estimate of high passage mortality was not due to a decrease in $\ln(R/S)$ over years 1959–1968 for the Snake stocks coincident with the construction of Snake River dams. Instead, it was due to an average increase in $\ln(R/S)$ for the Lower Columbia stocks (completely driven by the JDMF stock) that was unmatched by the Snake stocks.

Further investigation showed that the high $\ln(R/S)$ of 3.69 for 1968 was due to an estimated 23 spawners producing 882 recruits. This value of $\ln(R/S)$ was extreme, lying 2.72 standard deviations above the mean. The count of 23 spawners was arrived at by expanding a count of four redds in the middle fork of the John Day River (Beamesderfer et al. 1997). The estimate of 882 recruits from 1968 was largely the result of assuming that 82% of the estimated 920 adult returns in 1972 were four-year-olds (making them the offspring of the 1968 brood). This assumption was made because age data for recruitment were lacking in 1972 (Beamesderfer et al. 1997). Thus, it is possible that a counting error drove the high $\ln(R/S)$ for the JDMF 1968 observation, leading to a large error in the model parameter estimates. Other difficulties with the JDMF SR data were discussed in Lindsay et al. (1986). This is not to say that these observations are erroneous, but that any errors they contain can have a large effect on inferences about passage mortality.

The three observations also have high influence on the

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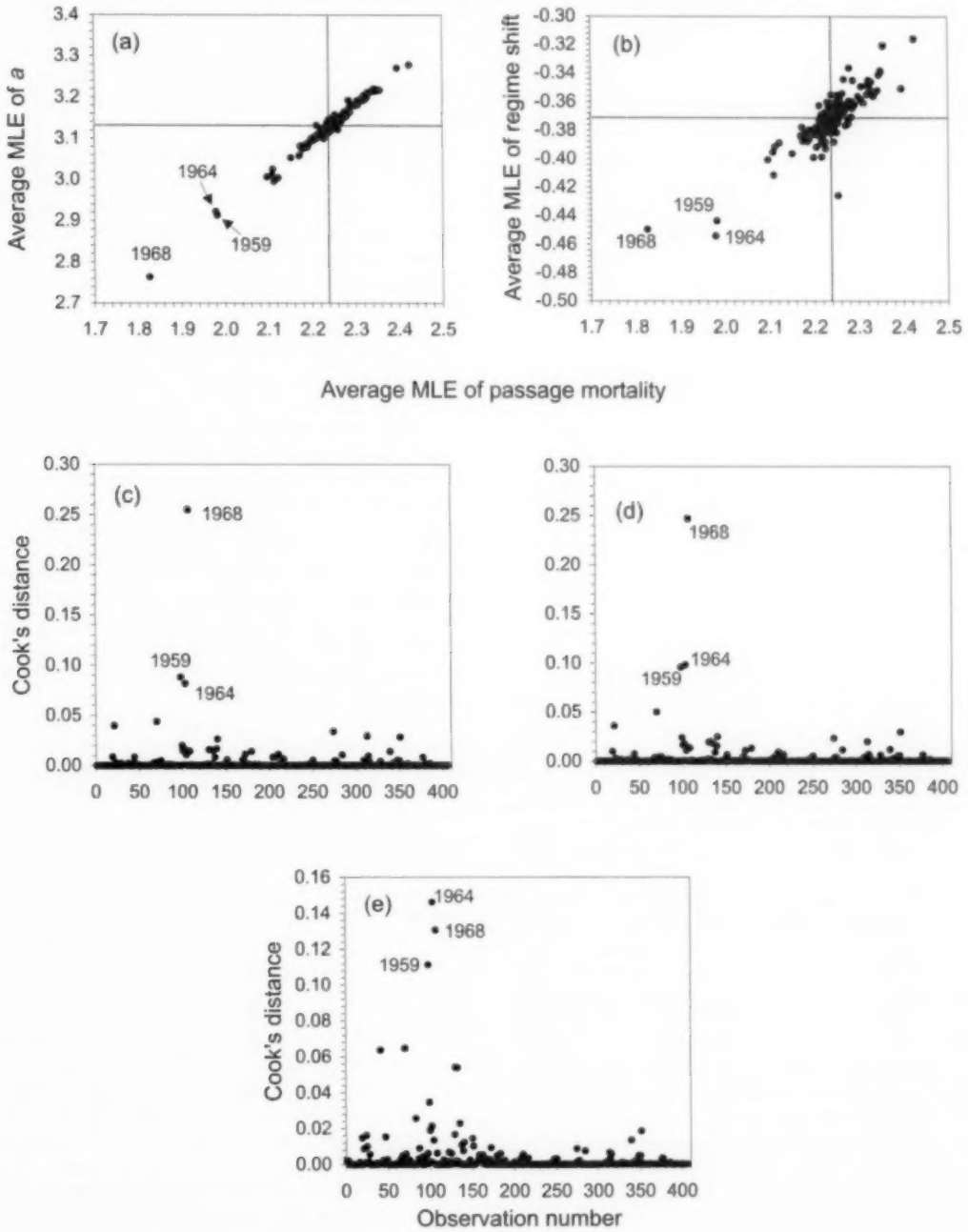
Fig. 1. (*a*) The maximum likelihood estimates (MLEs) of average intrinsic productivity and passage mortality obtained by deleting each of the $n = 406$ observations in turn. (*b*) The MLEs of the regime-shift parameter and passage mortality obtained by deleting each of the $n = 406$ observations in turn. The estimates with all the data present are given by the intersection of the cross hairs. Cook's distances are computed with respect to the average Ricker-*a* parameter (*c*), the average passage-mortality parameter (*d*), and the regime shift parameter (*e*). Note the relatively large influence of the 1959, 1964, and 1968 John Day Middle Fork observations.



Snake River intrinsic productivity and regime-shift estimates, because both these estimates are highly correlated with passage mortality, $r = 0.992$ and $r = 0.867$, respectively. These correlations are high because, in the estimation procedure, the Lower Columbia stocks are used to partition the total productivity of the stock (a fixed quantity) among

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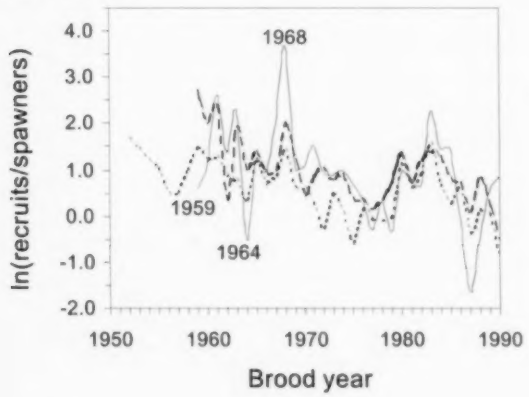
Table 2. DELTA model summary parameter estimates and standard errors for three different estimation procedures.

	Original regression		Bootstrap		AR(1) residual model	
	Estimate	SE	Mean estimate	SE	Estimate	SE
Passage mortality	2.24	0.83	2.24	0.76	2.21	1.02
Mean Ricker a	3.13	0.72	3.13	0.66	3.12	0.88
Mean Ricker b ($\times 10^3$)	1.22	0.16	1.23	0.14	1.31	0.15
Regime shift	–0.37	0.22	–0.37	0.20	–0.37	0.28
$\hat{\phi}$	na	na	na	na	0.40	0.06

Note: SE, standard error; AR(1), first-order autoregressive model.

Fig. 2. The John Day Middle Fork stock (solid line) showed a large increase in productivity during 1959–1968 compared with the other Lower Columbia stocks (broken line) and the Snake stocks (dotted line).



intrinsic productivity, passage survival, and regime shift. As the passage survival increases, achieving the same fixed total productivity of the Snake stocks requires the intrinsic productivity to decrease or the regime shift effect to decrease.

When all data were present, all summary parameters but the regime shift were significant at the 0.05 level, with absolute values of the estimates far exceeding standard errors (Table 2). Bootstrap bias estimates for the four summary parameter estimates were small: 0.001, 0.006, 5.91×10^{-6}, and –0.004 for the passage-mortality, average Ricker-a, average Ricker-b, and regime-shift parameters, respectively. The test for normality failed to reject the null hypothesis of normality of the error terms. The coefficient of correlation between the residuals and their expected value under normality was 0.9967, which was higher than the critical value of 0.9965 assuming a significance level of 0.05. The test of autocorrelation in the error term was significant at the 0.05 level, with an AR parameter estimate of $\hat{\phi} = 0.40$ (SE = 0.06). Including the AR parameter changed the maximum likelihood estimates (MLEs) of the summary parameters only slightly, but it did tend to increase standard errors (with the exception of the Ricker-b parameter) (Table 2).

Snake River ocean-type chinook

The estimate of transportation effectiveness, D, critical in determining the success of the transportation actions relative to the dam-breaching actions, was very sensitive to the deletion of brood year 1991 (Table 3). Cook's distance for the D parameter for brood year 1991 was 5.10, representing a change in the D estimate from 0.047 (SE = 0.065) to 0.194 (SE = 0.188) when 1991was omitted.

One of the reasons that the 1991 observation was so influential was that a large proportion of the offspring from that brood year were transported during their seaward migration (i.e., the value of the covariate P was relatively large in 1991), giving the observation high leverage. The proportion of fish from 1991 transported was 0.94 compared with a 1976–1991 average of 0.58. Observations with high leverage are highly influential when they are accompanied by unusual values of the response (Ryan 1997). The 1991 response was low regardless of our assumptions about the effectiveness of supplemented spawners, E. When $E = 0$, the response, $\ln(R/S) + M$, for 1991 was 0.688 compared with a 1980–1991 mean of 2.708 (SD = 0.790); when $E = 1$ (meaning supplemented spawners have the same reproductive success as wild ones), the 1991 response was 0.278 compared with a 1980–1991 mean of 2.330 (SD = 0.944). This low value of the 1991 response, coupled with the high leverage with respect to the fraction transported, P, almost guaranteed a small D estimate.

The estimates and their standard errors for the original model, bootstrap results, and autoregressive residual model are listed in Table 4. The bootstrap bias estimates were small: –0.0128, -1.03×10^{-6}, –0.0695, 6.90×10^{-5}, and 0.0247 for the estimates of a, b, E, g, and D, respectively. Although the bias of 0.025 for the transportation effectiveness, D, was large on a percentage basis (+53%), as I shall show in Discussion, this bias was insufficient to affect the conclusion on whether transportation should be halted. The correlation coefficient test failed to reject the null hypothesis of normality. The coefficient of correlation between the residuals and their expected value under normality was 0.981, which was higher than the critical value of 0.962 assuming a significance level of 0.05. There was no significant autocorrelation detected in the error term of the model; the MLE of the AR parameter was $\hat{\phi} = -0.17$ (SE = 0.23).

Discussion

The two examples from models currently used to assess passage survival and transportation of threatened chinook salmon runs in the Snake River Basin clearly show that certain observations can greatly influence parameter estimates and, in turn, the substantive inferences. For the Snake River ocean-type chinook salmon model, a single observation has enormous weight on the inference about the effectiveness of

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Table 3. Cook's distance for transport effectiveness, denoted d(D), and leverage for each observation.

	$\ln(R/S) - M$				
Brood year	$E = 0$	$E = 1$	P	d(D)	Leverage
1964	2.172	2.172	0.000	0.0021	0.09
1965	2.961	2.961	0.000	0.0000	0.07
1966	2.014	2.014	0.000	0.0007	0.11
1967	2.736	2.736	0.000	0.0001	0.17
1968	2.036	2.036	0.000	0.0671	0.57*
1969	3.229	3.229	0.000	0.0001	0.05
1970	3.459	3.459	0.000	0.0023	0.05
1971	2.852	2.852	0.000	0.0057	0.05
1972	3.777	3.777	0.000	0.0033	0.07
1973	3.116	3.116	0.000	0.0090	0.06
1974	4.392	4.392	0.000	0.0777	0.09
1975	3.418	3.418	0.000	0.0026	0.07
1976	3.860	3.860	0.075	0.0064	0.08
1977	3.566	3.566	0.129	0.0000	0.12
1978	3.233	3.233	0.121	0.0065	0.07
1979	3.898	3.898	0.346	0.0083	0.07
1980	3.580	3.580	0.683	0.0734	0.09
1981	2.642	2.642	0.569	0.0081	0.07
1982	2.688	2.688	0.633	0.0028	0.08
1983	3.300	3.223	0.585	0.0004	0.05
1984	3.772	3.511	0.683	0.0082	0.54*
1985	2.604	2.406	0.681	0.0012	0.05
1986	2.428	2.153	0.723	0.0014	0.05
1987	2.391	1.557	0.744	0.0029	0.48*
1988	2.955	2.606	0.650	0.0012	0.09
1989	2.353	1.685	0.772	0.0147	0.19
1990	3.096	1.630	0.922	0.0770	0.52*
1991	0.688	0.278	0.941	5.0975	0.85*

Note: R, recruits; S, spawners.
*The leverage exceeds the cutoff of 2 × (number of parameters)/(number of observations) = 0.36.

Table 4. FALLD model estimates and standard errors for different estimation procedures.

	Original regression		Bootstrap		AR(1) residual model	
	Estimate	SE	Mean estimate	SE	Estimate	SE
a	3.769	0.138	3.757	0.124	3.776	0.126
b ($\times 10^3$)	0.127	0.023	0.126	0.020	0.127	0.021
E	0.727	0.584	0.659	0.313	0.588	0.535
g	0.718	0.252	0.718	0.232	0.715	0.258
D	0.047	0.065	0.072	0.071	0.059	0.074
ϕ	na	na	na	na	–0.172	0.228

Note: SE, standard error; AR(1), first-order autoregressive model.

transportation. At the low D estimate of 0.047 (SE = 0.065), derived when all data were present, one would conclude that transportation should be halted, because average in-river survival, which is estimated at 0.22 (1976–1991), is higher than D, implying that in-river migrants have much higher survival rates than transported migrants. At the higher D estimate of 0.194, derived with the 1991 observation omitted, it is not clear whether transportation should be halted, because fish that were transported survived about equally well as fish that migrated in river. Thus Cook's distance flagged an observation that not only had a large effect on a parameter estimate, D, but also had a large effect on a very important practical inference.

Influential observations had a large effect on another important inference: the relative contribution of passage-mortality and climate regime shift effects to stream-type population declines. Using the DELTA model, PATH attempted to tease out how much loss of productivity was due to a regime-shift effect and how much was due to a passage-mortality effect. Scientists involved in PATH were divided over which played a larger role in population declines in the late 1970s. The DELTA model with all data present suggests

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that passage-mortality effects and regime-shift effects cut *R/S* by about 90 and 30%, respectively, suggesting that the effect of passage mortality was significantly greater than the effect of a regime shift ($\alpha = 0.10$). However, with the JDMF stock removed, these cuts in *R/S* were 66 and 49%, respectively, and the difference of these effects was not significant at the 0.10 level ($p = 0.72$).

Although identifying influential observations is important, it is the next step, determining how to treat them, that is most important. Should influential observations simply be removed from the Snake River chinook salmon data sets without further consideration? No. Removing an observation for consideration is only justifiable when it can be shown to be erroneous or unrepresentative of the process under study. In fact, because influential observations often lie at the extremes of the data set, they tend to be informative, revealing biological responses away from the typical levels of the independent variables, and thereby providing an opportunity to gain scientific understanding.

If influential observations are not omitted, why detect them at all? Because knowing whether conclusions hinge on a single observation (or small subset of observations) helps us portray uncertainty more accurately. Influence diagnostics can be every bit as relevant to making inferences as confidence intervals or standard errors. Although FALLD yields a fairly precise estimate of transportation effectiveness—$D = 0.047$ (SE = 0.065), indicating that transportation should be immediately halted—the Cook's diagnostic demonstrates that this inference (though it may be correct) is highly dependent on a single observation, making it extremely dubious. Some diagnostic of influence was needed here to portray the high uncertainty of the conclusion that juvenile transportation was harmful. This influence analysis brings the problem clearly into focus: to learn whether transportation aids juvenile salmon, transportation must be experimentally manipulated to produce contrast in the fraction of fish transported, *P*. Without such contrast, the single 1991 observation of low $\ln(R/S)$ associated with high fraction transported, *P*, could be viewed as a coincidence.

Influence diagnostics present only one suite of model checks next to checks of bias of estimates and the standard regression assumptions of normality, constant variance, and independence of errors. Clearly, biases and violations of these usual assumptions can be important. But in the case of the PATH models, the effects of biases of MLEs and assumption violations on inferences were negligible compared with the effects of influential observations. For example, adjusting for the bias in the estimate of *D* did not change the conclusion that transportation of Snake River fall chinook salmon decreases their overall productivity, but omitting the single 1991 observation did.

The influential observations flagged in the Columbia River chinook salmon data sets may be mistakes (arising from observation or transcription error) or real. Even when real, their identification is crucial, because they can adversely affect the substantive inferences of an analysis. For example, in the spring chinook salmon analysis, if the exceptional (influential) JDMF spring chinook salmon data is due to an unidentified anomaly specific to the JDMF instead of to an effect that influences all spring chinook salmon stocks in the analysis (e.g., rotenone treatments of the JDMF spawning bed in 1966 (Lindsay et al. 1986)), then the model improperly interprets the anomaly as an indication of higher passage mortality for Snake River spring chinook salmon stocks. Whether they are mistakes or real, influential observations should be identified and their effects on substantive inferences clearly stated. As the examples from Snake River chinook salmon SR models demonstrate, this step, most often missing from statistical analysis in fisheries and ecological investigations, can be crucial in properly tempering the inferences drawn from non-experimental data. As Efron and Tibshirani (1993) point out, statistics is the science of bringing together a number of small pieces of information to get an informative result. Investigators should be worried when just one piece of information (a single observation or small subset of observations) largely determines the answer to an important scientific or management question.

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A comparison of gamma and lognormal maximum likelihood estimators in a sequential population analysis

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Abstract: We analyze the model used to assess most major commercial marine fish populations, namely, sequential population analysis (SPA). This model estimates population abundance by combining catch-at-age data with research surveys or commercial catch per unit effort indices of abundance. We examine two maximum likelihood estimators of SPA parameters. These estimators are based on assuming that the stock-size indices are from lognormal or gamma distributions. Using simulations, we find that both types of estimators can have significant biases; however, our results indicate that it is preferable to use the gamma model, because it tends to have lower bias and variability, even when the true distribution of the stock-size indices is lognormal.

Résumé : On trouvera ici un examen de l'analyse séquentielle de population (SPA), le modèle qui est utilisé pour évaluer la plupart des populations de poissons marins d'intérêt commercial. Ce type de modèle estime l'abondance de la population en combinant des données sur la capture à chacun des âges à des indices d'abondance basés sur des inventaires scientifiques ou sur le succès de la pêche commerciale par unité d'effort. Deux estimateurs de vraisemblance maximale des paramètres de la SPA ont été comparés; les deux estimateurs supposent que les indices de densité des stocks sont tirés de distributions lognormales ou gamma. Des simulations montrent que les deux types d'estimateurs peuvent introduire des erreurs systématiques importantes; cependant, d'après ces résultats, il est préférable d'utiliser le modèle gamma car il introduit moins de biais et de variation, même lorsque la véritable distribution des indices de densité des stocks est lognormale.

[Traduit par la Rédaction]

Introduction

Sequential population analysis (SPA) is an important model used to provide abundance information for many major commercial fisheries worldwide (Shepherd 1988). This model is so named because it sequentially combines commercial catches from all fish born in a given year, and uses research-survey estimates of relative abundance (indices) or commercial catch rates to estimate the present and past absolute fish stock abundance (numbers at age). SPA is the standard method used in the management of fish populations by both international organizations, e.g., ICES (International Council for the Exploration of the Sea) and NAFO (Northwest Atlantic Fisheries Organization), and national governments, e.g., Canada and the U.S.A. Versions of this method are also used to analyze populations of seals (Roff and Bowen 1983), whales (Cooke 1985), and terrestrial mammals (McCullough 1979). There are serious known biases in the method presently used that have led to overestimation of abundance and overexploitation of some populations (ICES 1991; Cook et al. 1991). Even so, SPA has received relatively little statistical investigation.

The standard method of estimating SPAs is to use nonlinear least squares with transformed survey indices. Transformations are used so that model errors appear homogeneously distributed; however, another reason is that nonlinear least squares is relatively easy to use. A log transformation in conjunction with a normal-error assumption is commonly used, which is equivalent to assuming that the untransformed survey indices have a lognormal distribution. However, with the exception of Patterson (1999), the appropriate distribution for modeling the survey indices has received little attention. Part of the reason for the lack of statistical attention is that many researchers examine model residuals; they feel that, if serious problems exist with the stochastic assumptions, then these problems should be apparent in the residuals. A second conjecture is that less obvious violations in distributional assumptions have negligible effects on estimates and inferences, although Patterson (1999) demonstrates otherwise in an example. Both of these assumptions require verification. We focus on the second assumption in this paper.

The purpose of this paper is to explore the potential sensitivity of SPAs to the assumed distribution of survey indices. A log transformation is commonly used, and is appropriate when the model variability of the survey indices is proportional to the square of their mean (constant coefficient of variation (CV)), although other variance models may be more appropriate for some data sets. We explore SPA estimation for two constant CV stochastic models: the lognormal and the

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gamma. In practice, it is usually difficult to diagnose which of these distributions is the more appropriate one to use (see Atkinson 1982; McCullagh and Nelder 1989). It is often assumed that estimates and inferences are similar for either distribution and that, in practice, it does not matter whether the lognormal or gamma distribution is used. We investigate the bias and precision of maximum likelihood estimates (MLEs) of some SPA parameters for these two distributions using simulation studies. As well, we investigate the bias and precision when the distribution is mis-specified, that is, the change in bias and precision resulting from using lognormal MLEs with gamma-distributed survey indices, and vice versa. We also investigate the accuracy of a method for constructing confidence intervals. Our simulations are based on six real fish populations: Labrador cod, Southern Grand Bank cod, Eastern Scotian shelf cod, Browns Bank cod, Scotian Shelf pollock, and Southern Gulf of St. Lawrence herring.

Materials and methods

SPA can be thought of as "a family of methods for converting catch-at-age data into [population] numbers at age" (Mohn and Cook 1993). Megrey (1989) gives a description of many methods and models used in stock assessments, including SPA. We examine a formulation of SPA that is commonly used for North Atlantic fish stocks (Gavaris 1988; Hilborn and Walters 1992; Mohn and Cook 1993).

We consider a simple cohort model for a population with A age-classes ($a = 1,\ldots,A$) observed over Y years ($y = 1,\ldots,Y$). Let n_{ay} be the unknown number of fish in the population at the beginning of the year. The primary purpose of a SPA is to estimate the survivors in year Y; that is, the $n_{1Y},\ldots,n_{AY}$. The underlying population dynamics model for the SPA formulation we consider is

$$(1) \qquad n_{ay} = n_{a+1y+1}\exp(m) + c_{ay}\exp(m/2)$$

where c_{ay} is the number of age a fish caught by the fishery in year y and m is the annual natural mortality rate. This equation can be used to compute all population numbers (there are $A \times Y$ of these) from the population numbers in the last age and year (there are $A + Y - 1$ of these). We assume that the c and m are known without error. These are standard assumptions and have been used in the assessments of the six stocks we consider in our simulations. Equation 1 is based on the assumption that the commercial catches are taken halfway through the year. This approximation is from Pope (1972) and gives good results; Mertz and Myers (1996) show how eq. 1 can be easily modified to include a seasonal pattern of catches. We denote the survivors up to age A in year Y as the vector $\mathbf{n}_Y$. All n_{Ay}, $y = 1,\ldots,Y-1$, are usually expressed in terms of survivors, using nonlinear constraints (see Gavaris 1988; Myers and Cadigan 1995); however, we simply estimate them. Let $\mathbf{n}_A$ be a vector with elements $n_{A1},\ldots,n_{AY}$. Although not constraining $\mathbf{n}_A$ adds considerably more parameters to be estimated, the estimation is relatively easy, because the population-dynamics model is linear in the parameter vector $\mathbf{n} = [\mathbf{n}_A, \mathbf{n}_Y]$.

Statistical model and estimation

Gamma and lognormal MLEs for the unknown SPA parameters, $\mathbf{n}$, are presented in this section. Survey indices and catches are used to estimate $\mathbf{n}$. We stochastically model the survey indices. In most of the examples we consider, the surveys involve stratified random sampling using a trawl, and the indices are the mean number of fish per tow. It is common to assume that the survey indices reflect only an age-dependent proportion of stock abundance. This is because, among other reasons, the trawl is size-selective. Another common assumption for survey indices is that their SPA model variance is proportional to the square of their mean, that is, constant CV. Using these assumptions, the first two moments of our stochastic SPA model for the random survey indices (R) are

$$(2) \qquad E(R_{ay}) = \mu_{ay} = q_a n_{ay}$$

and

$$(3) \qquad \mathrm{Var}(R_{ay}) = k\mu_{ay}^2$$

where q_a is an age-specific survey-catchability coefficient. Let $\mathbf{q}$ be a vector with elements $q_1,\ldots,q_A$. The CV is $\sqrt{k}$. We explore maximum likelihood estimation of the $\mathbf{n}$ and $\mathbf{q}$ based on two distributions that are commonly used for constant CV stochastic models, namely, the gamma and lognormal distributions (see McCullagh and Nelder 1989).

Gamma SPA MLEs

Consider a gamma-distributed random variable $R > 0$ with $\mu = E(R)$, and let r be a realization of R. The probability density function (PDF) for R is

$$p(r;\mu,\phi) = r^{-1}\Gamma(\phi)^{-1}\left(\frac{\phi r}{\mu}\right)^{\phi}\exp[-\phi r/\mu]$$

where $\phi > 0$. For this distribution $\mathrm{Var}(R) = \mu^2/\phi$, so that $k = 1/\phi$ in eq. 3. The gamma log-likelihood for $\mathbf{n}$, $\mathbf{q}$, and ϕ is

$$\begin{aligned} L_G &= L_G(\mathbf{n},\mathbf{q},\phi\,|\,\{R_{ay} = r_{ay}\}) \\ &= AY[\phi\log(\phi) - \log\Gamma(\phi)] \\ &\quad + \phi\sum_{ay}\{\log(r_{ay}/\mu_{ay}) - r_{ay}/\mu_{ay}\} - \sum_{ay}\log r_{ay} \end{aligned}$$

where $\mu_{ay} = \mu_{ay}(\mathbf{n},\mathbf{q})$. MLEs for $\mathbf{n}$ and $\mathbf{q}$ are those that maximize L_G.

It is easy to show that the MLE for q_a when $\mathbf{n}$ is known is

$$\tilde{q}_a = \tilde{q}_a(\mathbf{n}) = Y^{-1}\sum_y \frac{r_{ay}}{n_{ay}}$$

Note that $\tilde{q}_a$ is unbiased for q_a. The MLE for ϕ has to be obtained numerically. McCullagh and Nelder (1989) do not recommend using the ϕ MLE for several reasons. They favor using the moment estimator

$$\hat{\phi} = \sum_{ay}\frac{[(r_{ay} - \mu_{ay})/\mu_{ay}]^2}{n - p}$$

The estimator of ϕ we use (denoted as $\tilde{\phi}$) is different from $\hat{\phi}$ only in that the $n - p$ term is replaced by n to be consistent with the lognormal variance parameter MLE (see next section). We use $\tilde{q}_a(\mathbf{n})$ and $\tilde{\phi}(\mathbf{n})$, to reduce the number of parameters that must be numerically estimated to just $\mathbf{n}$. We find the MLE for $\mathbf{n}$ using the Newton–Raphson method. The first order derivatives of $L_G(\mathbf{n}, \tilde{\mathbf{q}}, \tilde{\phi}\,|\,\{R_{ay} = r_{ay}\})$ are not difficult to calculate. We calculate $H(\mathbf{n}) = \partial^2 L_G(\cdot)/\partial\mathbf{n}\,\partial\mathbf{n}'$ by finite differences. Estimates of $\mathrm{Var}(\hat{\mathbf{n}})$ were obtained as the diagonal elements of $-H^{-1}(\hat{\mathbf{n}})$.

Lognormal SPA MLEs

The PDF for the lognormal distribution is

$$p(r;\mu,\sigma^2) = r^{-1}\sigma^{-1}(2\pi)^{-0.5}\exp\{-(\log r - \mu^*)^2/2\sigma^2\}$$

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Table 1. Fish populations analyzed.

Population	Unit area	Source	Ages	Years
Labrador cod	2J3KL	Bishop et al. 1993	3–12	1978–1992
Southern Grand Bank cod	3Ps	Bishop et al. 1994	3–12	1978–1993
Eastern Scotian Shelf cod	4VsW	Mohn and MacEachern 1993	3–15	1971–1992
Browns Bank cod	4X	Gavaris et al. 1994	3–10	1970–1992
Scotian Shelf pollock	4VWX	Trippel and Brown 1993	2–12	1974–1992
Southern Gulf of St. Lawrence herring	4T	Claytor et al. 1992	3–10	1978–1991

Note: Ages and years refer to minimum–maximum ages and first–last years used in the sequential population analysis.

Table 2. Number of simulations with large estimates of survivors.

	Survey distribution							
	CV = 0.5				CV = 1			
	Lognormal		Gamma		Lognormal		Gamma	
Stock	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE
3Ps cod	—	—	—	—	—	2	23	2
2J3KL cod	—	—	—	—	—	—	—	—
4VsW cod	—	—	—	—	—	—	—	—
4X cod	1	1	1	1	24	42	61	14
4T herring	—	—	—	—	—	—	27	16
4VWX pollock	—	—	—	—	17	34	42	12
Total	1	1	1	1	41	78	153	44

Note: GMLE and LMLE denote gamma and lognormal maximum likelihood estimators respectively.

where $\sigma > 0$ and $\mu^* = \log(\mu) - \sigma^2/2$. $\text{Log}(R)$ is normally distributed with mean μ^* and variance σ^2. For this distribution,

$$\text{Var}(R) = \exp(\mu^*)^2\exp(\sigma^2)\{\exp(\sigma^2) - 1\} = k\mu^2$$

where $k = \exp(\sigma^2) - 1$ in eq. 3. The log-likelihood for $\mathbf{n}$, $\mathbf{q}$, and σ is

$$L_N = L_N(\mathbf{n},\mathbf{q},\sigma^2|\{R_{ay} = r_{ay}\})$$

$$= -AY\log(2\pi\sigma^2)/2$$

$$- \sum_{ay}(\log r_{ay} - \mu^*_{ay})^2 / 2\sigma^2 - \sum_{ay}\log r_{ay}$$

It is easy to show that the MLE for $q_a^* = q_a \exp(-\sigma^2/2)$ when $\mathbf{n}$ is known is given by

$$\log(\tilde{q}_a^*) = \log[\tilde{q}_a^*(\mathbf{n})] = Y^{-1}\sum_y\{\log(r_{ay}) - \log(n_{ay})\}$$

This is an unbiased estimator of $\log(q_a^*)$. Also, the MLE for σ^2 is given by

$$\tilde{\sigma}^2 = \tilde{\sigma}^2(\mathbf{n}) = \frac{\Sigma_{ay}\{\log(r_{ay}) - \log(\tilde{\mu}_{ay})\}^2}{AY}$$

where $\tilde{\mu}_{ay} = \tilde{q}_a^* n_{ay}$. For known $\mathbf{n}$, $\tilde{\sigma}^2$ is a biased estimator of σ^2, and the bias is $-\sigma^2/Y$. This bias is of a similar order to the bias in $\hat{\phi}$. It may be more appropriate to use the bias corrected estimator of σ^2; however, this does not affect the estimation of $\mathbf{n}$, which is the main focus of this paper. The MLE of q_a can easily be obtained using $\tilde{q}_a^*$ and $\tilde{\sigma}^2$. We use $\tilde{q}_a^*$ and $\tilde{\sigma}^2$ to reduce the number of parameters to estimate, just as for the gamma case. We also use the same method to estimate $\text{Var}(\hat{\mathbf{n}})$.

Simulations

In this section, we describe the simulation procedures we use to compare gamma and lognormal MLEs of SPA parameters. We consider six fish stocks on which to base our simulations. We use published time series of catch at age and published estimates of n_{aY} ($a = 1,\ldots,A$), n_{Ay} ($y = 1,\ldots,Y-1$), and q_a, to generate random survey indices at age, using eqs. 1, 2, and 3. We generate 1000 sets of surveys assuming gamma or lognormal errors (2000 in total), and estimate $\mathbf{n}$ twice for each set using gamma and lognormal MLEs. The only difference between gamma and lognormal simulations is the distribution used to generate the random survey data. We generate three sets of simulated indices, with CVs of 0.25, 0.5, and 1. These values cover the range of SPA CVs that are common in stock assessments. We use the simulations to estimate the bias and other distributional characteristics of the gamma and lognormal MLEs of $\mathbf{n}$.

The six fish stocks we consider are all from the Northwest Atlantic (see Table 1). The population parameters are taken from the referenced assessment documents for each stock. We only consider SPAs up to the early 1990s, because commercial fishing moratoria and environmental anomalies after this period complicate the analysis of several of these stocks. We use an annual mortality rate, m, of 0.2, to randomly generate survey data in all our simulation analyses. The same value for m is used in estimation. Documents containing the data used in our analysis can be obtained from the Canadian Stock Assessment Secretariat, Fisheries and Oceans (Station 1256), 200 Kent Street, Ottawa, ON K1A 0E6, Canada, or the Northwest Atlantic Fisheries Organization, P.O. Box 638, Dartmouth, NS B2Y 3Y9, Canada.

Results

In some simulations, particularly when the CV = 1, the parameter estimates were implausibly large. For example, with one of the pollock simulated data sets, the gamma MLE of total survivors converged after 29 iterations and was 3.09×10^{13}; however, after eight iterations, the log-likelihood was within 3.4 units of the maximum but the survivors totalled only 1.6×10^5, which is relatively close to the population total of 9.2×10^4. This is a case where the simulated data

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Fig. 1. Standardized biases for lognormal (solid lines) and gamma (dotted lines) maximum likelihood estimators of the sequential population analysis survivors (n_{aY}, $a = 1,\ldots,A$). Panels on the left show biases for lognormal-simulated surveys (coefficient of variation (CV) = 0.5) and panels on the right show biases for gamma-simulated surveys (CV = 0.5). Each row of panels represents a particular fish stock: (*a*) 3Ps cod, (*b*) 2J3KL cod, (*c*) 4VsW cod, (*d*) 4X cod, (*e*) 4T herring, and (*f*) 4VWX pollock.

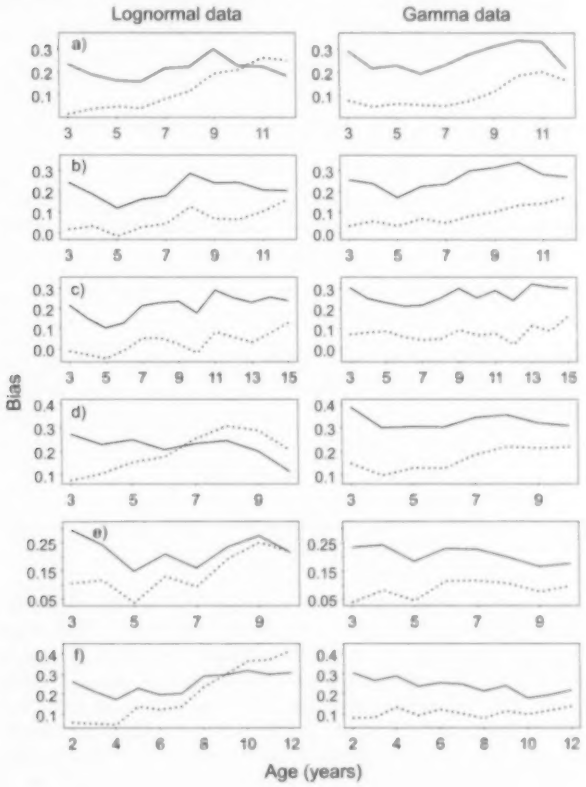
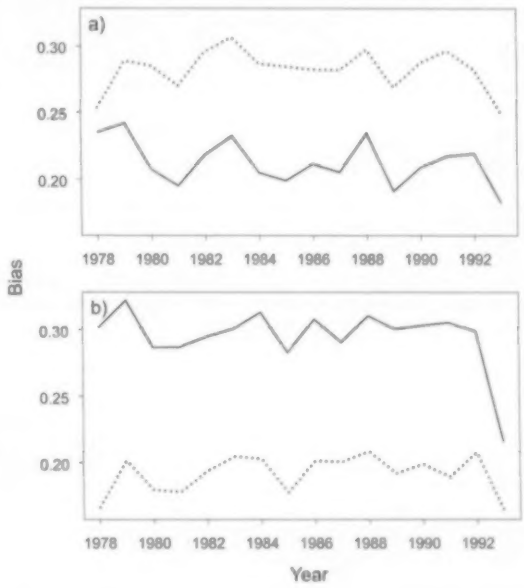


Fig. 2. Standardized biases for lognormal (solid lines) and gamma (dotted lines) maximum likelihood estimators of the sequential population analysis population numbers at the oldest age (n_{Ay}, $y = 1,\ldots,Y$). (*a*) Lognormal-simulated surveys (coefficient of variation (CV) = 0.5). (*b*) Gamma-simulated surveys (CV = 0.5).



are non-informative about how large the stock might be. We decided to remove these cases, to simulate the process that actually occurs during a stock assessment, that is, such data would not usually be considered in a stock assessment. We removed cases if the estimated total abundance of survivors exceeded 10× the population total abundance of survivors (i.e., $10 \times \Sigma_a n_{aY}$). Both the gamma and lognormal MLEs were removed if either one produced a large estimate of total survivors. The frequency of these large estimates is presented in Table 2. No large estimates were found when the CV = 0.25. When the CV = 1, the trend over all stocks was to obtain more large estimates when the error distribution was wrong, and this occurred more for lognormal MLEs than for gamma MLEs. We examine the consequences of not eliminating the large estimates at the end of this section.

Another problem for the remaining pollock simulations with CV = 1 was that negative variance estimates occurred three times. This happened twice for gamma MLEs and once for lognormal MLEs. Negative variances were always associated with very low estimates of stock size. The corresponding likelihood surfaces were very flat, and these simulations represent data sets that are not informative about how low stock size might be. These cases produced almost singular Hessian matrices that, when inverted, led to incorrect negative variance estimates. These simulations were also removed from further analysis. Note that negative variance estimates were also common in the simulations that produced unreasonably large estimates of stock size.

We focus on the relative performance of gamma and lognormal MLEs of the unknown SPA survivors, $\mathbf{n}_Y$. Let $\hat{\mathbf{n}}_Y^G$ denote the gamma MLEs and let $\hat{\mathbf{n}}_Y^L$ denote the lognormal MLEs. Standardized biases (see eq. 4 below) obtained from the CV = 0.5 simulations for $\hat{\mathbf{n}}_Y^L$ and $\hat{\mathbf{n}}_Y^G$ are presented in Fig. 1. The standardization is the same for both $\hat{\mathbf{n}}_Y^G$ and $\hat{\mathbf{n}}_Y^L$; it is

$$(4) \qquad B_s(\hat{\mathbf{n}}_Y) = \frac{\text{ave}(\hat{\mathbf{n}}_Y) - \mathbf{n}_Y}{\text{SD}_{\text{ave}}}$$

where $\text{ave}(\hat{\mathbf{n}}_Y)$ is the average of the simulated $\hat{\mathbf{n}}_Y$s,

$$\text{SD}_{\text{ave}} = [\{\text{Var}(\hat{\mathbf{n}}_Y^L) + \text{Var}(\hat{\mathbf{n}}_Y^G)\} / 2]^{1/2},$$

and $\text{Var}(\hat{\mathbf{n}})$ is the variance of $\hat{\mathbf{n}}$ calculated over the simulated estimates. If the only difference in the simulated distributions of $\hat{\mathbf{n}}_Y^G$ and $\hat{\mathbf{n}}_Y^L$ is a location shift, then SD_{ave} is simply a combined estimate of the standard deviation. The same SD_{ave} is used for both $\hat{\mathbf{n}}_Y^G$ and $\hat{\mathbf{n}}_Y^L$, so that differences in standardized bias are not the consequence of differences in precision; however, a different standardization is used in each panel in Fig. 1 and for each age. We examine the precision of estimators later. Note that the biases in Fig. 1 tend to be midway between the biases we found in the CV = 0.25 and CV = 1 simulations. These results demonstrate that $B_s(\hat{\mathbf{n}}_Y^G)$ is often less than $B_s(\hat{\mathbf{n}}_Y^L)$, even when the survey distribution is lognormal. This was always the case when the CV = 0.25.

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Table 3. Relative bias (%), relative median bias (%), and relative standard deviations (%) for maximum likelihood estimators (MLEs)

	Survey distribution											
	Relative bias (%)								Relative median bias (%)			
	CV = 0.25				CV = 1				CV = 0.25			
	Lognormal		Gamma		Lognormal		Gamma		Lognormal		Gamma	
Stock	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE
3Ps cod (108.6)	2	0	2	1	35	17	98	21	2	–0	2	0
2J3KL cod (335.8)	2	0	3	1	27	6	67	19	1	–1	2	0
4VsW cod (50.6)	1	–0	2	0	21	–1	52	12	1	–1	1	–1
4X cod (21.8)	3	1	3	0	63	88	175	41	1	–1	2	–1
4T herring (1940.6)	3	1	2	0	36	28	136	42	1	0	1	–1

Note: GMLE and LMLE denote gamma and lognormal MLEs. Values in parentheses are population total survivors (millions).

Table 4. Relative bias (%), relative median bias (%), and relative standard deviations (%) for maximum likelihood estimators (MLEs)

	Survey distribution											
	Relative bias (%)								Relative median bias (%)			
	CV = 0.25				CV = 1				CV = 0.25			
	Lognormal		Gamma		Lognormal		Gamma		Lognormal		Gamma	
Stock	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE
3Ps cod (0.10)	–1	1	–1	1	–10	10	–26	4	–2	0	–2	–0
2J3KL cod (0.11)	–1	1	–1	0	–9	13	–23	3	–1	1	–2	–0
4VsW cod (0.46)	–0	1	–1	1	–8	17	–20	5	–1	1	–1	1
4X cod (0.47)	–1	1	–1	2	–15	–1	–30	9	–1	1	–2	1
4T herring (0.07)	–1	0	–0	1	–7	3	–14	7	–1	–0	–1	1

Note: GMLE and LMLE denote gamma and lognormal MLEs. Values in parentheses are the population total exploitation rate.

The biases for n_{A_y} (i.e., $\hat{\mathbf{n}}_A$) when $y < Y$ are similar in magnitude to the bias of $\hat{\mathbf{n}}_{AY}$, which is shown at the right end-point in each of the panels in Fig. 1. We present complete bias results for 3Ps cod in Fig. 2. The results demonstrate that $|B_s(\hat{\mathbf{n}}_A^G)| - |B_s(\hat{\mathbf{n}}_A^L)|$ when the survey distribution is lognormal is less than $|B_s(\hat{\mathbf{n}}_A^L)| - |B_s(\hat{\mathbf{n}}_A^G)|$ when the survey distribution is gamma.

The estimated total abundance of survivors (i.e., $\Sigma_a n_{aY} = n_{+Y}$) is a useful quantity to examine in more detail. In Table 3, we present the bias, median bias, and simulation standard deviations for this quantity. The biases and standard deviations in Table 3 are expressed as percentages of total population (true) abundances (n_{+Y}), which are also shown in this table; for example, the average relative percentage bias is given by

$$B_r(\hat{n}_{+Y}) = 100 \times \left(\frac{\text{ave}(\hat{n}_{+Y}) - n_{+Y}}{n_{+Y}} \right)$$

The median (med) bias is computed by replacing ave(·) with med(·). The results for CV = 0.5 are intermediate between those presented in Table 3.

The relative bias results in Table 3 demonstrate that $|B_r(\hat{n}_{+Y}^G)|$ is usually less than $|B_r(\hat{n}_{+Y}^L)|$. Also in Table 3, we show that the median biases for both $\hat{n}_{+Y}^L$ and $\hat{n}_{+Y}^G$ tend to be less than the average biases, which is to be expected, because the distributions of $\hat{n}_{+Y}^L$ and $\hat{n}_{+Y}^G$ are right-skewed. The median biases demonstrate that, at least 50% of the time, the SPA estimates of n_{+Y} are closer to the population value than the average biases suggest. The median bias of $\hat{n}_{+Y}^G$ tends to be negative quite frequently, whereas the bias of $\hat{n}_{+Y}^L$ is always positive. Hence, we conclude that, more often than not, gamma MLEs will underestimate survivors and lognormal MLEs will overestimate survivors. The relative standard deviation results in Table 3 demonstrate that, when the CV = 0.25, both $\hat{n}_{+Y}^L$ and $\hat{n}_{+Y}^G$ appear to be equally precise, and there is little loss in precision if the nominal MLE distribution is mis-specified. This is not the case when the CV = 1; however, our results show that a greater loss in precision is incurred by using $\hat{\mathbf{n}}_Y^L$ with gamma surveys than by using $\hat{\mathbf{n}}_Y^G$ with lognormal surveys.

Another useful quantity to examine in detail is the total exploitation rate in the last year, that is, $\Sigma_a c_{aY} / \Sigma_a n_{aY} = F_Y$. This is the fraction of the initial stock in year Y removed by the fishery. Exploitation rates are commonly used to assess the sustainability of a fishery. Average and median biases for the total exploitation rate are presented in Table 4. The results when the CV = 1 suggest that, at least 50% of the time, lognormal MLEs underestimate exploitation rates by a considerably greater amount than gamma MLEs do. A disadvantage of gamma MLEs is that the standard deviations of $\hat{F}_Y^G$ (see Table 4) tend to be 40% greater than the standard deviations of $\hat{F}_Y^L$ for lognormal surveys with CV = 1, and 20% greater than the standard deviations of $\hat{F}_Y^L$ for gamma surveys. The effect of this is that for CV = 1, $\text{MSE}(\hat{F}_Y^G) > \text{MSE}(\hat{F}_Y^L)$ even when the surveys are gamma distributed. This is largely because gamma MLEs overestimate exploitation rates more than lognormal MLEs. For all six stocks and for lognormal data with CV = 1, the average 10th percentiles of $\hat{F}_Y^L - F_Y$ and $\hat{F}_Y^G - F_Y$ are –52 and –49% of F_Y, respectively. For

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of total survivors.

Relative standard deviations (%)											
CV= 1				CV = 0.25				CV = 1			
Lognormal		Gamma		Lognormal		Gamma		Lognormal		Gamma	
LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE
21	–1	55	9	12	12	12	12	84	120	216	59
18	–3	48	11	11	11	11	11	52	47	88	54
14	–9	40	5	10	10	10	9	43	40	69	44
26	11	68	9	15	14	15	14	143	356	540	240
15	6	37	6	13	13	13	13	81	83	478	166

of the total exploitation rate in the last sequential population analysis year.

Relative standard deviations (%)											
CV = 1				CV = 0.25				CV = 1			
Lognormal		Gamma		Lognormal		Gamma		Lognormal		Gamma	
LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE	LMLE	GMLE
–17	1	–35	–9	12	12	11	12	38	54	46	57
–15	4	–32	–10	11	11	11	11	35	51	42	53
–12	9	–28	–4	9	10	9	9	31	45	40	46
–21	–10	–41	–8	14	14	14	14	42	57	49	70
–13	–6	–27	–6	13	13	13	13	43	51	59	64

gamma surveys, the average 10th percentiles of $\hat{F}_Y^L - F_Y$ and $\hat{F}_Y^G - F_Y$ are –68 and –50% of F_Y, respectively. However, the 90th percentiles of $\hat{F}_Y^L - F_Y$ and $\hat{F}_Y^G - F_Y$ are 36 and 70% of F_Y, respectively, for lognormal surveys and 33 and 73% of F_Y, respectively, for gamma surveys.

An important aspect of SPA is the "correctness" of inferences, that is, how well do confidence intervals (CIs) cover population values. Of specific interest to us is how CIs based on the lognormal- or gamma-distribution assumptions compare, especially when the distribution is mis-specified. We investigate this using CIs based on a normal approximation for the distribution of MLEs. Let V^{-1} be the observed information matrix based on $\ln(\mathbf{n})$ parameters. We nominally assume that

$$(5) \qquad \frac{\ln(\hat{\mathbf{n}}) - \ln(\mathbf{n})}{V^{1/2}}$$

has a standard normal distribution, and use this statistic to construct CIs. These intervals are commonly used for SPA inference, and give a simple way to compare the potential accuracy of inferences for gamma and lognormal MLEs. In our simulations, we count the number of times population values exceed CIs. Lower CIs are of particular importance for fisheries management. In Fig. 3, we plot the observed proportion of times in the CV = 0.5 simulations that population values for survivors were less than the 95% lower confidence limit. The limit based on eq. 5 appears too large, because population values are smaller than this limit with a frequency greater than the nominal 5% value. Nonetheless, the relative inaccuracy of the gamma-based lower limit compared with the lognormal limit when the surveys are lognormal is less than the inaccuracy of the lognormal-based lower limit compared with the gamma limit when the surveys are gamma. Similar results were obtained when the CV = 1. When the CV = 0.25 (results not shown), the limit based on the gamma assumption is more accurate than the limit based on the lognormal assumption, even when the distribution is mis-specified.

In the above analyses, we eliminated estimates that were implausibly high, that is, 10 times the truth. Our general results were robust to changes to this procedure. If fewer very large estimates were removed, the bias of the lognormal estimator increased greatly, while if estimates five times the truth were eliminated, the bias was reduced. In both cases, the gamma MLEs seemed preferable.

Discussion

We have found that assumptions about the distribution of research-survey indices in the method most commonly used to estimate the abundance of marine fish populations in the North Atlantic have a large effect on the bias of important parameters for fisheries managers. The lognormal distribution is commonly used for SPA estimation and inference; however, the gamma distribution appears preferable. This is because gamma MLEs have less bias even when the true distribution of survey indices is lognormal or, at least, the increase in bias when using gamma MLEs with lognormal-distributed surveys is less than the increase in bias when using lognormal MLEs with gamma-distributed surveys. Our results also suggest that, for estimating population size, the loss in precision using

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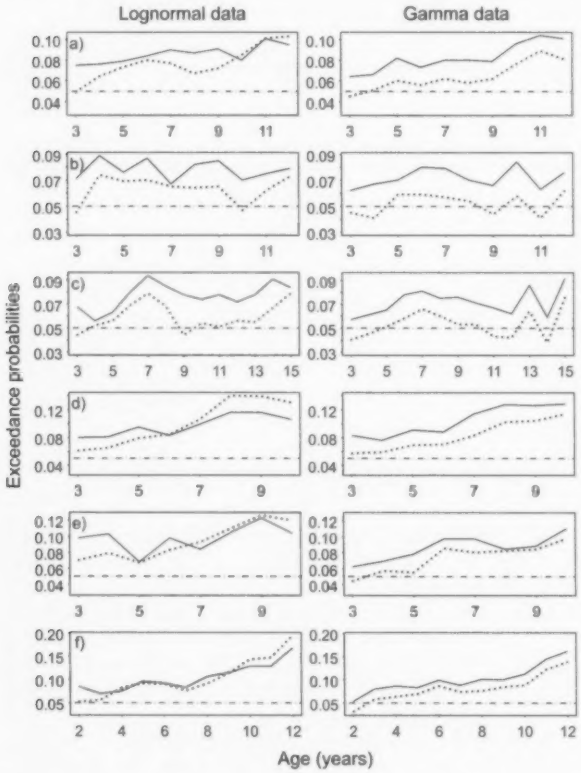
Fig. 3. Simulation probabilities that sequential population analysis survivors (n_{aY}, $a = 1,\ldots, A$) are less than lower confidence limits based on lognormal (solid lines) and gamma (dotted lines) maximum likelihood estimators. Panels on the left show biases for lognormal-simulated surveys (coefficient of variation (CV) = 0.5). Panels on the right show biases for gamma-simulated surveys (CV = 0.5). Each row of panels represents a particular fish stock: (*a*) 3Ps cod, (*b*) 2J3KL cod, (*c*) 4VsW cod, (*d*) 4X cod, (*e*) 4T herring, and (*f*) 4VWX pollock. The nominal probability of exceeding the confidence limit is shown as a broken line.



lognormal MLEs with gamma-distributed surveys tends to be greater than the loss in precision using gamma MLEs with lognormal-distributed surveys. Our results also suggest that more accurate and robust lower CIs for population size may be obtained using the gamma-distribution assumption rather than the lognormal-distribution assumption.

Our conclusions agree with those presented by Firth (1988), who showed that the loss in asymptotic efficiency of gamma MLEs when errors are lognormally distributed is less than the loss associated with lognormal MLEs when the errors are gamma distributed. Our results are also consistent with Myers and Pepin (1990), who found that methods that rely on the lognormal assumption may produce large biases and loss of efficiency, if there are even small deviations from the assumptions.

One deficiency of gamma MLEs of SPA exploitation rates compared with lognormal MLEs is that gamma MLEs were found to be less precise than lognormal MLEs, even when the surveys were gamma distributed. This could be serious, because exploitation rates often play an important role in setting commercial-fishery catch quotas; that is, often quotas are selected that give a low predicted exploitation rate. Our results show that the difference in precision is because gamma MLEs tend to overestimate exploitation rates more than lognormal MLEs, that is, the probability of getting a large estimate of the exploitation rate in the last SPA year is considerably greater for gamma MLEs than for lognormal MLEs. This is a less serious conservation error than underestimating exploitation rates and overestimating stock size, which is more of a problem for lognormal than gamma MLEs. Consequently, the decreased precision of gamma MLEs for estimating exploitation rates compared with lognormal MLEs is not a good reason to favor the use of lognormal MLEs.

Some of our assumptions may be violated for some data sets. In particular, there may be errors in the observed commercial catches, and natural mortality may vary over time and age. Violation of either of these assumptions may create biases and increase estimation error variance (Lapointe et al. 1989; Clark 1999). Trends in unreported catch or discarding can have disastrous consequences in a collapsing fishery (Myers et al. 1997). Another problem is that survey indices may not be independent within years (Myers and Cadigan 1995) for some stocks. We have limited our analyses to the model defined by eqs. 2 and 3, because it accounts for a major source of variability (surveys, see Rivard 1989), and it is desirable for our analyses to be applicable to fish stock assessment methods that are commonly used, at least for North Atlantic stocks. It also seems unlikely that lognormal MLEs may be preferable to gamma MLEs, because some other part of the SPA model is mis-specified. Nonetheless, the estimation biases we report may be small for some stocks compared with the biases that can result from using incorrect catches and wrong values for natural mortality.

Our approach to SPA estimation is somewhat nonstandard in North Atlantic assessments, in that we treat as unknown both the survivors ($\mathbf{n}_Y$) and the numbers in the oldest age-class of the cohort model ($\mathbf{n}_A$). Typically, the latter numbers are approximated using constraints on their fishing mortalities. This reduces the number of parameters to estimate, but also complicates estimation. We feel that our results will apply when constraints on fishing mortalities are used, providing the constraints are good approximations of reality. Otherwise the choice of error distribution may be a minor problem in terms of the biases caused by incorrectly modeling the $\mathbf{n}_A$. In this respect, our results also suggest that, when the survey SPA errors are not too large (CV $\leq$ 0.5), then it is possible to estimate the $\mathbf{n}_A$ and $\mathbf{n}_Y$ with reasonable accuracy, and we recommend doing this at least as a diagnostic check for the suitability of fishing-mortality constraints.

More complicated and possibly more realistic stochastic assumptions are also used for modeling catch-at-age data. Examples are found in Deriso et al. (1985) and Gudmundsson (1994). These methods all involve stochastic models for commercial catches. The catch models usually express c_{ay} in terms of population numbers, using a commercial fishery selectivity model. These methods may also allow for errors in assumptions about the annual natural mortality rate, m. Quite often these methods incorporate additional information, like the effort expended by the commercial fleet to obtain catches. The applicability of our results to these other methods is difficult

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to predict, however our comments in the previous two paragraphs also apply to these more complicated approaches; for example, if the fishery-selectivity model is mis-specified, then the choice of survey SPA error distribution may be a minor problem compared with the effect of incorrectly modeling commercial-catch information.

Gamma and lognormal MLEs produce different estimates, essentially because both distributions give different weights to the deviations between observations and predictions. An alternative approach is to use quasi-likelihood methods (see McCullagh and Nelder 1989), as they only use information on the first two moments.

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Effects of fishing on a protogynous hermaphrodite

Paul R. Armsworth

Abstract: Population dynamic models and simulations are analysed for a harvested reef fish species that is a monandric, protogynous hermaphrodite. The models are applied to data for the coral trout *Plectropomus leopardus* (Pisces: Serranidae) on the Great Barrier Reef. One model examines the situation where sexual transition is induced by some exogenous behavioural mechanism, and another considers the case where transition is determined by some endogenous developmental schedule. The conclusions regarding the effects of fishing are common to both models, and the implementation of more efficient harvesting practices may not require a precise understanding of the mechanisms governing sexual transition.

Résumé : On trouvera ici une analyse de modèles et de simulations de la dynamique d'une population de poissons de récif hermaphrodites monandres et protogynes qui est soumise à pêche commerciale. Les modèles ont été appliqués à des données provenant d'une population du serranidé, *Plectropomus leopardus* (Pisces: Serranidae), du récif de la Grande Barrière. L'un des modèles examine la situation où la transition sexuelle est causée par un mécanisme comportemental exogène et l'autre celle où cette transition est déterminé par un calendrier de développement endogène. Les effets de la pêche calculés à l'aide des deux modèles sont semblables; il n'est donc probablement pas nécessaire de connaître de façon précise les mécanismes qui régissent la transition sexuelle pour mettre en place des pratiques de pêche plus efficaces.

[Traduit par la Rédaction]

Introduction

Many commercially important reef fish species are hermaphrodites. Fisheries theory developed for gonochoristic populations may not be applicable to these species (Bannerot et al. 1987; Russ 1991). Models and simulations that examine the effects of harvesting on a hermaphroditic fish are presented and these are applied to data for the coral trout or leopard coral grouper *Plectropomus leopardus* (Pisces: Serranidae). Coral trout are sequential hermaphrodites, in which the hermaphroditism is protogynous (female–male) and monandric (males are only produced through sexual transition) (Thresher 1984; Ferreira 1995). The species is a long-lived, apex predator on the Great Barrier Reef. The burgeoning literature on *P. leopardus* serves as testament to its commercial importance in Australia. Coral trout comprise 30% of the total catch of the Queensland commercial line-fishing fleet, and the stocks support a booming, recreational line fishery (Ferreira and Russ 1994).

Two types of mechanism could induce sexual transition in hermaphroditic fishes. Either exogenous behavioural mechanisms could determine the timing of transition, such as the size of an individual relative to other members of its social group, or transition could be associated with some endogenous developmental schedule, such as absolute size or age. While exogenous mechanisms have been detected (Shapiro 1989; Ross 1990), endogenous mechanisms may still be important in some species (Thompson and Munro 1983; Buxton 1993) or may interact with exogenous factors. If fishing is size selective, then the consequences for population structure depend on mechanisms governing transition. If sex change is governed by social mechanisms, then transition would be expected to occur earlier in the life cycle when older, single-sex individuals are removed. This would cause the mean size and age of females to decrease for protogynous species and could cause a reduction in egg production. If sexual transition were driven by some fixed developmental schedule, the population could not compensate for impacts to its sex ratio. Selective fishing of older individuals would then lead to sex ratios that were shifted from their natural state; these would be female biased for protogynous hermaphrodites.

In the models and simulations, particular attention is paid to the importance of metapopulation concepts and fluctuating recruitment levels. As is conventional in reef fish ecology, "recruitment" refers here to recruitment to a local adult population following pelagic larval dispersal and does not refer to age of first vulnerability to gear. Harvesting causes a directional shift through parameter space. The consequences of fishing for population dynamics can be understood by examining the magnitude of the change in parameter values and comparing this with the deterministic and stochastic analyses. In the following section, the model formulation and the deterministic and stochastic dynamics are described. Then later, the effects of harvesting and the size of the movement through parameter space that results from fishing are considered.

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Population model

The population is structured into ω age-classes, $\mathbf{N} = (N_1,...,N_\omega)$, with reproduction occurring in a short annual spawning season. This is representative of coral trout behaviour on the Great Barrier Reef (Doherty et al. 1994; Samoilys and Squire 1994). The census time is set immediately before reproduction.

Each year-class is subdivided by sex, with $f_i = f_i(\mathbf{N})$ and $m_i = m_i(\mathbf{N})$ denoting the expected proportion of the ith year-class that is female and male, respectively. As hermaphroditism is protogynous, $f_i > m_i$ for younger age-classes. In Model 1, sexual transition is governed by an endogenous developmental schedule, and an individual female has a fixed probability of changing sex in each season; this probability is independent of the state of the population. Thus, the expected proportion of each age-class that is male and female is fixed, and f_i and m_i are treated as constants. In Model 2, sexual transition is dictated by social factors, and the probability that an individual female remains female throughout some season is assumed to be directly proportional to the mean age of the population. This probability is constrained to be less than or equal to 1. Thus, if fishing lowers the mean age of the population, the probability that a female will change sex at a given age increases. Both models are assumed to have the same proportions of males and females in equilibrium in the absence of fishing pressure.

The mortality rate is assumed to be density independent in year-classes 1 through $(\omega - 1)$. Let p_i denote the per capita survival rate of individuals in the ith cohort. Let ν_i and μ_i represent the fertility of females and males of age i, respectively, where fertility describes the rate of reproductive success and is assumed to be proportional to fecundity. Fecundity increases nonlinearly with size, and larger fish contribute disproportionately to the spawning stock; this is accommodated in the ν_i and μ_i values.

Reef fish populations are best modelled as metapopulations of interconnected local populations that communicate through dispersal of pelagic larvae (Sale 1998). This study focuses only on the dynamics of a single local population but explicitly considers its role within the context of the wider metapopulation. The population is both partially open and partially closed. Let ξ represent the external supply rate and a represent the proportion of individuals produced locally that successfully recruit back to their reef of origin.

Production in the local population depends on the number of encounters between males and females, weighted by the fertilities. A constant scaling factor that governs the rate of encounters between individuals from different age-classes is subsumed into the fertility measures. Therefore, P_{t+1}, the production in season $t + 1$, is

$$(1) \qquad P_{t+1} = \left(\sum_{i=1}^{\omega} \nu_i f_i N_{i_t}\right)\left(\sum_{i=1}^{\omega} \mu_i m_i N_{i_t}\right).$$

It could be argued that production should be scaled by some form of marriage function (Caswell 1989), which would relate the density of males and females to the rate of successful reproduction and describe the complex reproductive behaviour of coral trout (Samoilys 1997). However, I know of no studies on serranids that determine what form such a function would take. Dependence of production on the encounter rate between males and females is one of the simplest relationships that can be assumed.

The supply of larvae to the population in the spawning season at the beginning of year $t + 1$ is

$$(2) \qquad S_{t+1} = aP_{t+1} + \xi.$$

It is assumed that if population regulation occurs, then it occurs in the first year, that it is the per capita mortality rate of individuals in the settling (year 0) cohort that is density dependent, and that the density dependence is compensatory but not overcompensatory. Compensatory density dependence is modelled with a Beverton–Holt type function (Beverton and Holt 1957), with the relationship between supply and recruitment given by

$$(3) \qquad R_{t+1} = \frac{\alpha S_{t+1}}{1 + \beta S_{t+1}}$$

where α describes the survival rate at low densities and α/β determines the saturation limit. Thus, the population models become

$$(4) \qquad N_{1_{t+1}} = R_{t+1} \text{ and } N_{i+1_{t+1}} = p_i N_{i_t} \text{ for } i = 1,...,\omega - 1.$$

The supply–recruitment relationship is rather general, and the dynamics depend entirely on the choice of parameters. If $\beta = 0$, then the mortality rate prior to censusing in the first year is density independent. If β is positive but sufficiently small, then the mortality rate of the settling cohort would be effectively density independent in the majority of years and would be strongly affected by density dependence only in times of anomalously high supply. This may be the case for many local reef fish populations including those of epinepheline serranids (Letourneur et al. 1998).

In Appendix A, how the available data for *P. leopardus* are used in the application of the models is explained. The sensitivity of the conclusions to the model assumptions and the choice of parameter values is reviewed in the Discussion.

Analysis

For brevity, results are presented only for the case where $a \neq 0$ and $\beta \neq 0$. In other words, there is some degree of regular self-recruitment, and year 0 mortality is density dependent. Other cases can be dealt with in a similar fashion. Technical details of the analyses are provided in Appendix B.

In the absence of fishing, critical point solutions of eq. 4 are identical for both models. At equilibrium

$$(5) \qquad \mathbf{N}_{t+1} = \mathbf{N}_t \Rightarrow N_i = \prod_{j=0}^{i-1} p_j N_1 \quad \text{for } i = 2,...,\omega$$

$$P = \left(\sum_{i=1}^{\omega} \nu_i f_i \prod_{j=0}^{i-1} p_j\right)\left(\sum_{i=1}^{\omega} \mu_i m_i \prod_{j=0}^{i-1} p_j\right) N_1^2 = F_0 M_0 N_1^2$$

where

$$(6) \qquad F_0 = \sum_{i=1}^{\omega} \nu_i f_i \prod_{j=0}^{i-1} p_j \text{ and } M_0 = \sum_{i=1}^{\omega} \mu_i m_i \prod_{j=0}^{i-1} p_j$$

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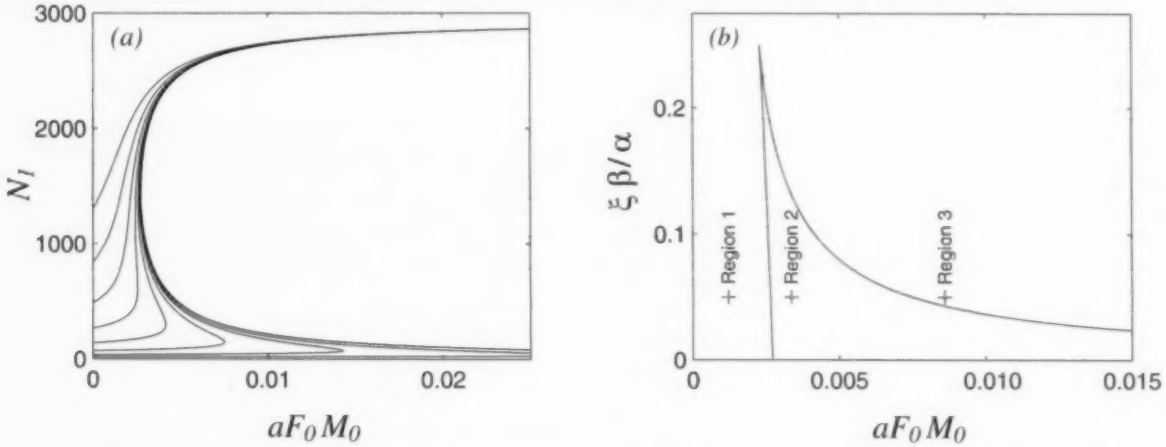
Fig. 1. All figures were produced using the data given in Appendix A. (*a*) Cross-sections of the equilibrium surface plotted against aF_0M_0 for $\xi\beta/\alpha$ = [0, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6], given in increasing order of vertical intercept; (*b*) projection of the outlines of the cusp onto the (aF_0M_0, $\xi\beta/\alpha$) plane. The curves separate parameter space into three regions.



(p_0 = 1 for notational convenience). These definitions adapt the net reproductive number from standard age-structured models (Cushing and Yicang 1994) and measure the expected lifetime reproductive contribution of individuals as females and males.

Critical point solutions for both models are given by roots of

$$(7)\quad g(N_1) = a\beta F_0 M_0 N_1^3 - a\alpha F_0 M_0 N_1^2 + (1+\beta\xi)N_1 - \alpha\xi = 0$$

(see Appendix B). This cubic has one or three positive roots. When fishing pressure is introduced, the f_i and m_i terms differ between the two models, and consequently, the F_0 and M_0 terms also differ. However, the cubic is unaffected by the additional nonlinearity in Model 2 because the mean age of the population is independent of the population size at equilibrium. Thus, eq. 7 still determines critical point solutions for Model 2.

The population dynamics for both models are characterised by a cusp catastrophe, in which the equilibrium surface is folded over on itself in parameter space (Drazin 1992). Such dynamics often arise when considering the interaction between nonlinear production terms with compensatory density dependence (Cushing 1996). Cross-sections of the equilibrium surface showing the fold are given in Fig. 1*a*. Double roots of the cubic determine the outlines of the fold, and the curves corresponding to such roots separate parameter space into three distinct regions (Fig. 1*b*).

The concept of source and sink dynamics (Thomas and Kunin 1999) proves helpful when distinguishing among regions of parameter space. Several definitions of source and sink dynamics are employed in the literature. Here, a source population is defined to be one in which the local rate of successful self-recruitment exceeds the local death rate; the inequality is reversed in a sink population. A source population is self-sustaining, but a sink population depends on external supply for its persistence.

Region 1 in Fig. 1*b* corresponds to dynamics possessing a single stable critical point solution that represents a sink population. Region 2 is characterised by the existence of three critical point solutions: two stable attractors separated by an unstable threshold. The lower stable critical point represents a sink state, and the larger represents a source state. Region 3 has a single stable equilibrium that represents a source state.

Local population dynamics appear to be more sensitive to the self-recruitment rate a than to the external supply rate ξ. Variations in a correspond to horizontal movements through parameter space in Fig. 1*b*, which can shift the population between regions that have markedly different qualitative dynamics.

Monte Carlo simulations

Rates of arrival of larvae at sites suitable for settlement are extremely variable. Monte Carlo simulations were designed to examine how the underlying deterministic dynamics of a local population might interact with this variability. Details regarding the construction of the simulations are given in Appendix B.

While critical point solutions are identical for both models in the absence of fishing, transient dynamics differ. However, the differences prove slight, and results from both models are almost identical for the same set of simulated a and ξ for the unfished case. What minor differences do arise result from fluctuations in recruitment that drive fluctuations in the mean age of the population in Model 2. These fluctuations in mean age feed back into local productivity via fluctuations in the sex ratio. However, the sex ratio never shifts far from that observed in the data to which both models are fitted.

Sink populations in Region 1 fluctuate around the single stable attractor for both models (Fig. 2*a*). The number of recruits joining the first year-class displays nearly perfect correlation with external supply (correlation coefficient ρ = 1.00 for the trajectory shown), and distortion of supply fluctuations due to density dependence is negligible.

Source populations in Region 3 also fluctuate around a single, globally stable attractor. Fluctuations are asymmetric, and perturbations that decrease population size are larger than those that increase population size (Fig. 2*b*). This is the opposite pattern to that displayed by both the a and ξ distributions (small values in most years with sporadic good years when the values obtained are much larger). Numerical ex-

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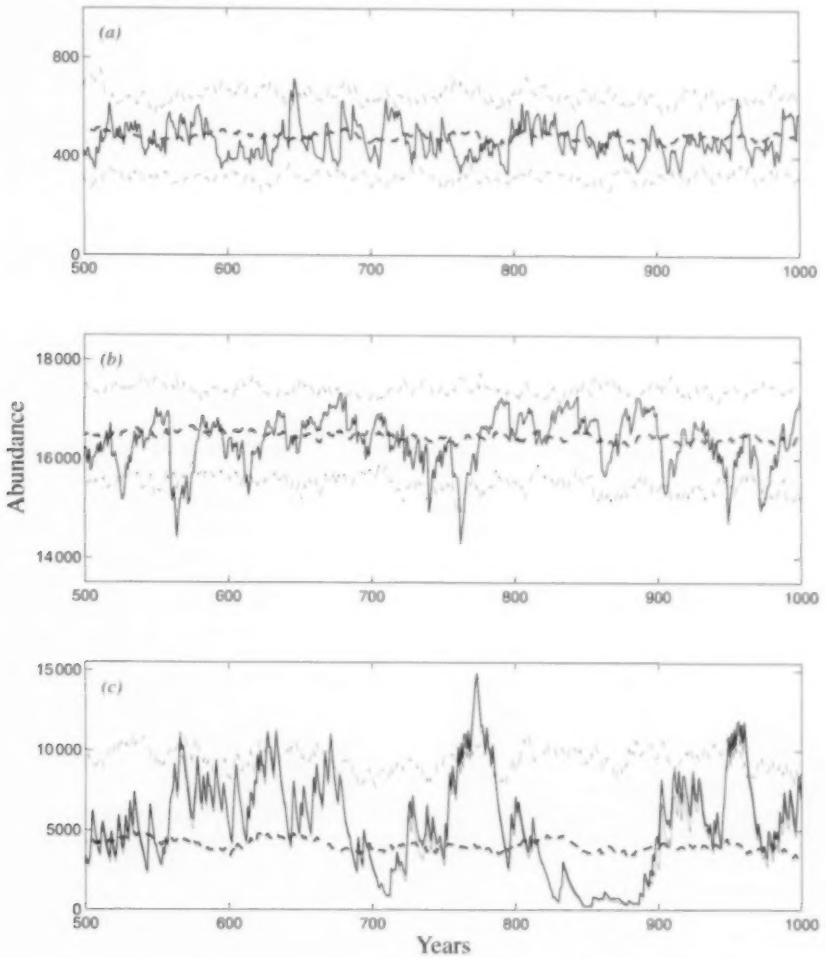
Fig. 2. Simulated population abundances (summed across all 14 cohorts) for Models 1 and 2. The thick broken line is mean abundance from 40 simulations for Model 1 and thin broken lines represent ±2 SD. A single realisation for both models is graphed with solid lines, and Model 1 is represented by the thicker of these lines. (*a*) Sink population data from Region 1 when variance is low (CV = $1/\sqrt{3}$ for a and ξ); (*b*) source population data from Region 3 when variance is low (CV = $1/\sqrt{3}$ for a and ξ); (*c*) population having two stable equilibria when variance is high (CV = 4 for a and ξ).



periments in which one variable is held constant indicate that the fluctuations in abundance are primarily driven by variations in self-recruitment rate a.

In the interior of Region 2, the dynamics show behaviour similar to that for the deterministic cusp when variance in a and ξ is low. The population converges on one of the two stable attractors (source or sink), and which is approached depends on the initial conditions. As variance is increased, fluctuations in a cause the population to shift sporadically between source and sink status (Fig. 2*c*).

More complex behaviour can arise in the region around the cusp curves themselves because of an interaction between recruitment fluctuations and the bifurcation behaviour of the models. For parameter values around the lower cusp curve, low initial population sizes lead to sink dynamics, but the population fluctuates around a region where the other two equilibria appear intermittently for larger initial population sizes. Variance in population size is greatly increased in this region, and the population fluctuates widely in response to fluctuations in a (Fig. 3). Eventually, a string of poor recruitment years leads to the sudden collapse of the population to permanent sink status. Similar dynamics arise around the upper cusp curve.

Effects of harvesting

Having described the different population dynamics, we can now examine the effects of fishing. The catch-per-unit-effort hypothesis (Hilborn and Walters 1992) is adopted and the effort level is denoted by E. Vector $\mathbf{q} = (q_1,\ldots,q_\omega)$ defines the catchability coefficients for each cohort. It is assumed that individuals do not become susceptible to capture until after the census time at the end of their first year and that fishing occurs after reproduction. The population dynamics under harvest are given by

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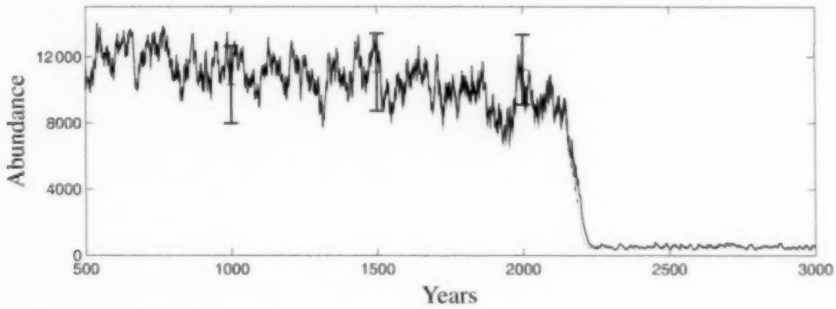
Fig. 3. Simulated population abundances (summed across all 14 cohorts) for Models 1 and 2 near the lower bifurcation with low variance (CV = $1/\sqrt{3}$ for a and ξ). A single realisation for both models is graphed with solid lines, and Model 1 is represented by the thicker of these lines. At t = [1000, 1500, 2000], the mean abundance ± 2 SD is shown for Model 1 for the subset of 60 simulations initialised in a source state that have not yet collapsed to sink status: n = [48, 42, 36], respectively. Collapses to sink status followed a string of poor self-recruitment years. After collapse, all populations remained in the sink state for the remainder of the simulation (5000 years).



$$N_{1,t+1} = R_{t+1} \text{ and } N_{i+1,t+1} = (p_i - q_i E)N_{i,t} \quad \text{for } i = 1,\ldots,\omega-1. \tag{8}$$

Both models have the same structure as eq. 4. Fishing mortality reduces the effective p_i values and thus also reduces the effective F_0M_0 value. This causes a shift to the left through parameter space that is parallel to the aF_0M_0 axis in Fig. 1*b*, the magnitude of which differs for the two models. This change in parameter values corresponds to a shift from source towards sink status. An increase in fishing mortality can take a population from a regime characterised by a single stable state through a region containing two stable states in which population size varies widely in response to varying supply rates. In summary, an increase in fishing effort in either model could result in a decrease in population abundance, a shift from source to sink status, an increase in variability in population size caused by fluctuations in supply rates, and the possibility of sudden, unpredictable collapse of seemingly stable stocks at some future time. An intensification of fishing need only result in the first of these four conclusions, and which ones are realised will depend on the natural state of the population and the extent of fishing mortality.

Gear selectivity

To assess the vulnerability of the population to selective gear, one must examine how F_0M_0 decreases when fishing mortality is introduced in each model. Cases where the fishery catches the same yield in overall biomass are compared. Greater effort is required to catch a fixed yield when relying on a highly selective strategy than is required when relying on a nondiscriminatory strategy. If the required yield is too large, then highly selective strategies will not be feasible. The optimal feasible selectivity strategy is defined as the one that causes the least reduction in F_0M_0 and hence incurs the least risk of overfishing while providing some specified yield.

Details concerning how the F_0M_0 values were computed for each model under harvest are given in Appendix A. Let (i, j) represent an exploitation strategy where the fishery targets the ith through jth cohorts where $i \in [1,\ldots,\omega]$ and $j \in [i,\ldots,\omega]$. Figure 4 illustrates the effect on F_0M_0 of harvesting an annual catch equivalent to 3.3% of virgin biomass when using different strategies. Each circle represents a feasible exploitation strategy, and the solid circle in the back corner represents the optimal strategy. The optimal strategy for both models is one that targets only the oldest fish in the population.

The sensitivity of the optimal selectivity strategy to the assumed growth schedule, the mean age of sexual transition, the survivorship rate, and the level of harvesting for both models was explored. A single parameter was varied each time, and the remaining parameters were fixed at their specified values. For a large region of parameter space centred upon the data for coral trout, the optimal strategy for both models lies in the back corner of the set of allowable strategies and involves targeting only older age-classes. An exception to this occurs for low survivorship probabilities. When the survivorship probabilities are particularly low, then the majority of the settling cohort are lost to natural mortality in the first few years of life. It can be optimal in this situation to harvest only younger individuals that would otherwise be lost to the fishery.

Fishing alters the population's age and sex structure for both models. Figure 5 compares scenarios where there is no fishing, where the gear is not selective and an annual catch equivalent to 2.75% of virgin biomass is harvested, and where the gear selectively targets the oldest (10+) cohorts and the same overall biomass is caught. Fishing strategies that target the oldest cohorts cause less disturbance to the natural sex structure of the population than less discriminatory strategies. Size-selective fishing also results in a greater overall mean age and a greater mean age of females than less selective fishing practices.

The most substantial differences between the two models arise in the consequences of fishing mortality for population structure. The number of individuals surviving to reach older age-classes decreases when fishing mortality is imposed. In Model 1, this has a disproportionate effect on the male life stages. Therefore, the sex ratio becomes female biased (Figs. 5*b* and 5*d*). In Model 2, decreased survivorship lowers the mean age of the population and triggers younger sexual transition. The number and size of females in the population decrease as a result, and the sex ratio becomes male biased because the population overcompensates for impacts to its sex ratio (Figs. 5*c* and 5*e*). For both models, changes in population structure are most pronounced for indiscriminate fishing practices.

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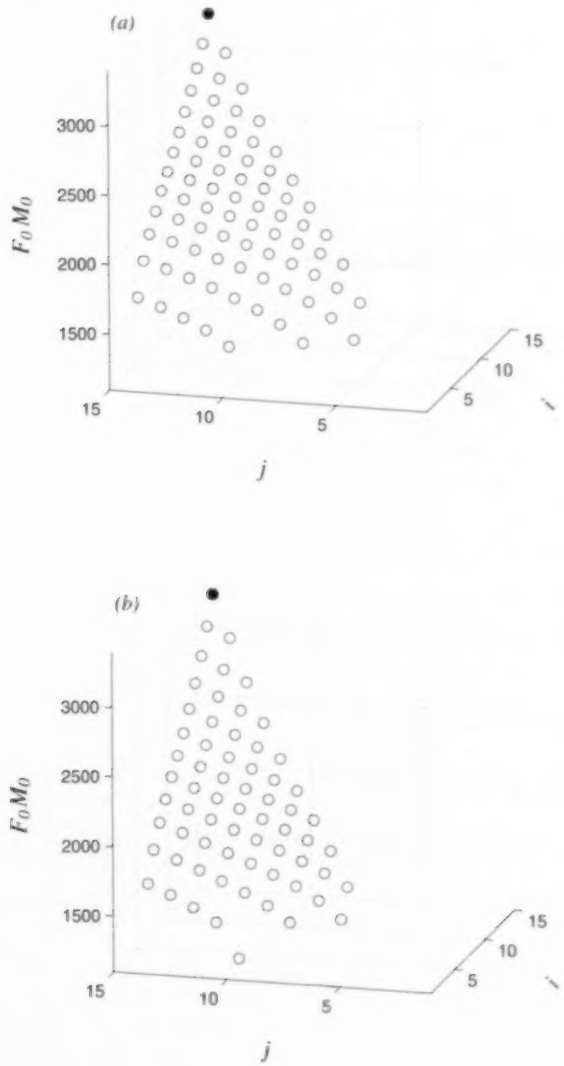
Fig. 4. Comparison of selectivity strategies when a fixed harvest is taken (equivalent to 3.3% of virgin biomass) for (*a*) Model 1 and (*b*) Model 2. Equal catchability of targeted cohorts is assumed. Circle (*i*, *j*) represents a feasible strategy that targets cohorts *i* through *j*, and the solid circle represents the optimal selectivity pattern.



Discussion

The deterministic models capture the essential dynamics of the situations under examination: those of partially open populations of protogynous hermaphrodites in which sexual transition responds to either endogenous or exogenous mechanisms (Models 1 and 2, respectively) and that are subjected to harvesting pressure. The resulting dynamics are characterised by a cusp catastrophe, one of the best understood of nonlinear phenomena (Drazin 1992) with well-studied implications for the management of natural systems (May 1977). Monte Carlo simulations reveal that the deterministic models perform well in regions of parameter space where they would be expected to be robust to perturbations, i.e., away from the bifurcations. Results from the deterministic analyses are not directly analogous to those from the stochastic simulations in the region around the bifurcations or when the variance in parameter values is high. Further modelling work examining more complex reproductive dynamics and more sophisticated mechanisms governing sexual transition would be worthwhile. The modelling framework presented here would provide a useful starting point for such analyses and for studies examining the effects of harvesting on other hermaphrodites.

Substantial uncertainty surrounds many of the processes involved, and one should consider any sensitivity to the assumptions made and the particular parameter values employed. The assumption regarding the density-dependent mortality in the first year of life can be relaxed and a similar analysis applies, although a source population grows without bound. If the mortality rate were characterised by overcompensatory density dependence or if other demographic rates were density dependent, then different dynamics could result.

The assumption that reproductive success is proportional to the number of encounters between males and females is one of the simplest about reproduction that could have been employed. It would be interesting to incorporate a more sophisticated marriage function (Caswell 1989) that represents the complex reproductive behaviour of coral trout (Samoilys 1997). However, provided there is some dependence of the per capita reproductive success of females on the number of males, then the population dynamics will be characterised by a threshold effect with multiple equilibria and similar conclusions will apply. Bannerot et al. (1987) used simulations to compare the vulnerability of protogynous hermaphrodites and gonochoristic species to overfishing under different assumptions about reproduction and concluded that hermaphrodites would be the more vulnerable as the likelihood of sperm limitation increased.

Uncertainty remains about many of the parameter values used such as the age-specific mortality rates and catchabilities, the rate of fertilisation success, and the rate of successful self-recruitment. This uncertainty can be accommodated because these processes are summarised by a single statistic, aF_0M_0. The population dynamics were examined for all possible values of this quantity. The conclusions regarding size-selective fishing proved robust when exploring a large region of parameter space centred on the data for coral trout.

Effects of harvesting

A general trend that appears in these and many other models is that fishing results in a shift from source status towards sink status. If a population were to lie in Region 3 when unexploited and external recruitment had not failed, then a shift to sink status could be reversed and the population would recover were fishing to cease. However, if a population were in Region 2 when unexploited or external recruitment failed, then a shift to sink status could be irrevocable. As indicated by the stochastic analyses, shifts to sink status can be sudden and dramatic, even for a fishery that had been sustainable for many years. This tends to occur

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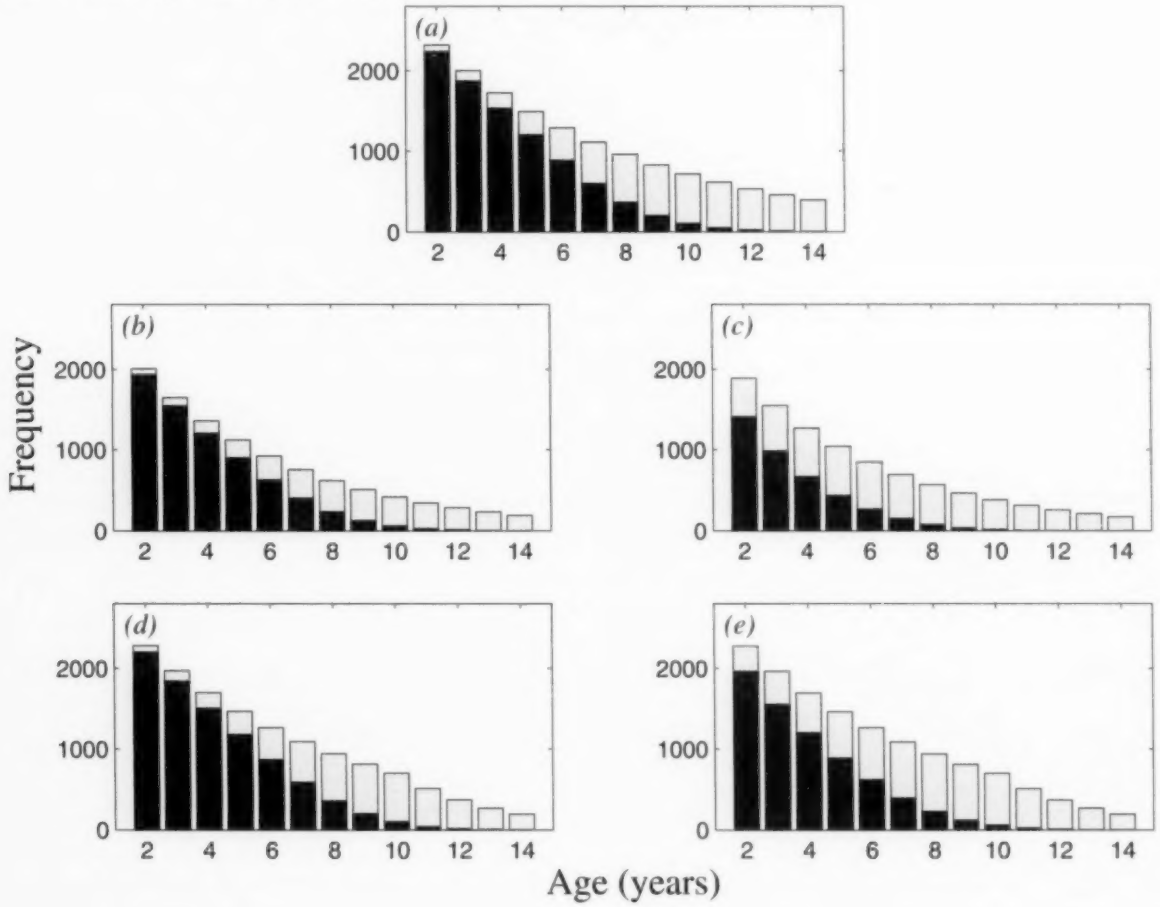
Fig. 5. Effect of different fishing strategies on population structure. Equal catchability of targeted cohorts is assumed. Shaded bars represent males and solid bars females. (*a*) Natural state of the source population; same for both models. Sex ratio of males to females m:f = 1:1.70, mean age $\overline{W}^* = 5.26$, mean female age $\overline{W}_f^* = 4.25$, and $F_0M_0 = 3274$. (*b* and *c*) New equilibrial population structure reached when annually removing 2.75% of the total virgin biomass through nonselective harvesting of all age-classes 1,..., ω for (*b*) Model 1 (m:f = 1:2.13, $\overline{W} = 4.67$, $\overline{W}_f = 4.05$, $F_0M_0 = 1816$) and (*c*) Model 2 (m:f = 1:0.72, $\overline{W} = 4.62$, $\overline{W}_f = 3.58$, $F_0M_0 = 1511$). (*d* and *e*) New equilibrial population structure reached when removing the same amount of biomass annually through selective harvesting targeting of cohorts 10,..., ω for (*d*) Model 1 (m:f = 1:1.92, $\overline{W} = 4.98$, $\overline{W}_f = 4.23$, $F_0M_0 = 2840$) and (*e*) Model 2 (m:f = 1:1.09, $\overline{W} = 4.97$, $\overline{W}_f = 4.00$, $F_0M_0 = 2766$).



when the population is subjected to the cumulative stress of fishing and a run of poor recruitment years. A shift from source to sink status results in a local population becoming entirely dependent on external supply. If that supply comes from outside the jurisdictional boundaries of the management agency, this shift results in the effective loss of sovereignty over the stock because persistence is contingent on events elsewhere.

The collapse of source populations can have metapopulation-wide consequences. Persistence of metapopulations, which could comprise entire stocks in large geographic regions, is contingent on the persistence of their local source populations or subregions. The fewer of these sources that remain, the less resilient a metapopulation is to catastrophic disturbance and the greater the risk of total regional stock collapse.

Gear selectivity

In both models, fishing alters both the sex ratio and the age structure of the population. Selectively targeting only older individuals results in a greater mean age in the population and has less effect on the sex ratio than less selective harvesting. The change in aF_0M_0 that corresponds to an increase in fishing effort is lowest when only older age-classes are targeted, and hence, selective fishing can minimise the risk of overfishing while supplying the same overall catch.

On the Great Barrier Reef, the minimum legal size for catching coral trout is 38 cm, which corresponds to an age of from 2 to 5 years. The precise mechanisms governing sexual transition in this species are unknown and will be difficult to detect (Ferreira and Russ 1995). The models suggest that a larger minimum size limit could be more efficient for

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both transition mechanisms. Thus, more efficient management practices could be implemented now without requiring precise knowledge of the sex-changing mechanisms. However, further research into the reproductive behaviour of coral trout would improve our understanding of where any thresholds between source and sink status might lie.

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Appendix A

The maximum age for *P. leopardus* was taken to be ω = 14 years (Ferreira and Russ 1994). The age of first reproduction is set at 2 years (Ferreira 1995). The expected proportions of males and females in each cohort in the absence of fishing are taken from Ferreira's (1995) Lizard Island data. For convenience, transitional individuals were classified as male. A least squares regression was used to fit these data to a curve of the form

$$\text{(A1)} \quad \frac{1 - \tanh(c_1(t - c_2))}{2}$$

($c_1 = 0.32$, $c_2 = 7.24$) and the fitted data were employed in the model applications; c_2 determines the mean time of sexual transition and c_1 determines the dispersion about this mean. Age-specific probabilities of remaining female for a given age in the absence of fishing can be computed:

$$\text{(A2)} \quad T_1 = f_1 \text{ and } T_i = f_i/f_{i-1} \quad \text{for } i = 2,\ldots,\omega.$$

Ferreira and Russ (1994) approximated the growth rate of *P. leopardus* in the Central Great Barrier Reef with Schnute's (1981) generic equation. For the specific data, Schnute's formula was found to be equivalent to the von Bertalanffy growth curve for length at time t:

$$\text{(A3)} \quad L(t) = L_\infty(1 - e^{-K(t-t_0)})$$

where L_∞ = 52.2 cm is the asymptotic length, K = 0.354 is the growth coefficient, and t_0 = –0.766 is the hypothetical age at which length is zero. From this length-based approximation, an equivalent expression can be obtained for the weight of individuals of a given age.

Per capita survivorship coefficients were taken as constant throughout the life cycle. The mortality rate is taken from the

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catch-per-unit-effort data of Russ et al. (1998) (only available for years 6 through 9): $p_i = 0.863$ for $i = 1,\ldots,\omega - 1$.

Sadovy (1996) reviewed the available data on fecundity and gave an approximation for the female fecundity F of groupers as a function of body length L:

$$F = 0.0129L^{3.03}. \quad \text{(A4)}$$

For want of alternative data, the fecundity of males is assumed proportional to the fecundity of females of the same age. The results are not sensitive to the constant of proportionality chosen.

Estimates of parameters α and β are not available. To produce the figures, the probability of individuals surviving settlement when settling in low densities was taken as $\alpha = 0.5$. Parameter β was chosen so that source population size was 17 500, which is roughly comparable with estimated population sizes at Lizard Island (Zeller and Russ 2000). Source population size was chosen because there is evidence from other species to suggest a significant degree of self-recruitment to Lizard Island (Jones et al. 1999).

Good data are not available for the rate of supply to reefs from external populations or for the proportion of locally produced propagules that survive to successfully recruit back to their reef of origin. However, the regions of parameter space in which these values must lie can be deduced from what is known of the ecology of coral trout and from dimensional analyses. The dynamics for the full range of plausible parameter values are examined in the deterministic model and simulations. In the section on the effect of harvesting, $\xi = \alpha/(20\beta)$ and $a = 2.63 \times 10^{-6}$ (corresponding to 35% larval mortality rate per day for a 25-day pelagic larval stage, with one in every eight surviving larvae returning to their reef of origin).

For the unfished case, F_0M_0 is directly proportional to the equivalent quantity when fecundities are used in place of fertilities in eq. 6. The constant of proportionality is chosen so that the parameters correspond to the right-hand cross in Fig. 1*b*.

When a fixed yield in overall biomass is to be harvested, the required effort can be computed under the assumption of equal catchability of targeted cohorts and from the equivalent expression to eq. A3 for weight. Therefore, new survivorship coefficients can be obtained, $\hat{p} = p - qE$, and F_0M_0 values under harvest obtained for Model 1.

New $\hat{f}_i$ and $\hat{m}_i$ values must also be computed for Model 2 because harvesting changes the mean age of the population. Let $\overline{\mathbf{W}}$ and $\overline{\mathbf{W}}^*$ denote the mean ages of the harvested and unharvested populations, respectively. In equilibrium,

$$\overline{\mathbf{W}} = \frac{\sum_{i=1}^{\omega} i \prod_{j=0}^{i-1} \hat{p}_j}{\sum_{i=1}^{\omega} \prod_{j=0}^{i-1} \hat{p}_j} \quad \text{(A5)}$$

A similar expression holds for $\overline{\mathbf{W}}^*$. When fishing pressure is applied in Model 2, the probabilities of remaining female at some age become

$$T_1 = f_1 \overline{\mathbf{W}}/\overline{\mathbf{W}}^* \text{ and } T_i = (f_i \overline{\mathbf{W}})/(f_{i-1}\mathbf{W}^*) \quad \text{for } i = 2,\ldots,\omega. \quad \text{(A6)}$$

Then

$$\hat{f}_i = f_i(\overline{\mathbf{W}}/\overline{\mathbf{W}}^*)^i \quad \text{for } i = 1,\ldots,\omega \quad \text{(A7)}$$

with the constraint imposed that $\hat{f}_i$ must be less than or equal to 1. Hence, the new $\hat{m}_i$ values, and consequently the new F_0M_0 values, can be obtained.

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Appendix B

At equilibrium, solutions of eq. 4 for both models solve

$$N_1 = \frac{\alpha(aF_0M_0N_1^2 + \xi)}{1 + \beta(aF_0M_0N_1^2 + \xi)} \quad \text{(B1)}$$

which gives eq. 7 on rearranging.

The cusp curves in Fig. 1*b* describe parameter sets for which eq. 7 has double roots. Let

$$N_1 = Q + \frac{\alpha}{3\beta} \Rightarrow 0 = Q^3 - uQ + v \quad \text{(B2)}$$

where

$$u = \frac{\alpha^2}{3\beta^2} - \frac{1 + \beta\xi}{a\beta F_0M_0}$$

and

$$v = \frac{-2\alpha^3}{27\beta^3} + \frac{\alpha(1 - 2\xi\beta)}{3a\beta^2F_0M_0}.$$

Turning points of the cubic solve

$$0 = 3Q^2 - u \Rightarrow Q = \sqrt{u/3}. \quad \text{(B3)}$$

Double roots are found by solving eqs. B2 and B3 simultaneously to give

$$v^2 = 4u^3/27. \quad \text{(B4)}$$

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Substituting for u and v in eq. B4 and rearranging gives

$$(B5)\quad 0 = (aF_0M_0)^2 + \frac{8\beta^2\xi^2 - 20\beta\xi - 1}{4\alpha^2\xi}(aF_0M_0) + \frac{\beta(1+\beta\xi)^3}{\alpha^4\xi}.$$

The projection of the cusp outline onto the $(aF_0M_0, \alpha\xi/\beta)$ plane shown in Fig. 1*b* was drawn by solving eq. B5 as a quadratic in aF_0M_0.

Stability properties of each equilibrium are governed by the magnitude of the dominant eigenvalue(s) of the Jacobian. The magnitude of the dominant eigenvalue must be less than 1 for a critical point to be stable. The Jacobian for Model 1 has a particular, well-studied form. If G_i denotes the growth function for the ith cohort, then:

$$(B6)\quad \frac{\partial G_i}{\partial N_j} = p_j \quad \text{if } j = i-1 \quad \text{and} \quad \frac{\partial G_i}{\partial N_j} = 0 \quad \text{otherwise.}$$

For the first age-class:

$$(B7)\quad \frac{\partial R}{\partial N_j} = \frac{dR}{dP}\frac{\partial P}{\partial N_j} = \frac{dR}{dP}\left(\nu_j f_j \sum_{i=1}^{\omega}\mu_i m_i N_i + \mu_j m_j \sum_{i=1}^{\omega}\nu_i f_i N_i\right)$$

Let R' denote the derivative of R with respect to P, $F = \Sigma_{i=1}^{\omega}\nu_i f_i N_i$, $M = \Sigma_{i=1}^{\omega}\mu_i m_i N_i$, and $\mathcal{T}_j = \nu_j f_j M + \mu_j m_j F$. The Jacobian is of the form

$$(B8)\quad \begin{pmatrix} \mathcal{T}_1R' & \mathcal{T}_2R' & \cdots & \cdots & \mathcal{T}_\omega R' \\ p_1 & 0 & \cdots & \cdots & 0 \\ 0 & p_2 & \ddots & & \vdots \\ \vdots & \ddots & \ddots & & \vdots \\ \vdots & & \ddots & \ddots & \vdots \\ 0 & \cdots & 0 & p_{\omega-1} & 0 \end{pmatrix}.$$

A compensatory density dependence is considered, the supply–recruitment relationship is not backwards bending, and R' is always positive. Therefore, the Jacobian is nonnegative and takes the form of the familiar Leslie matrix. Nonnegativity of a matrix implies that its dominant eigenvalue is nonnegative (Caswell 1989).

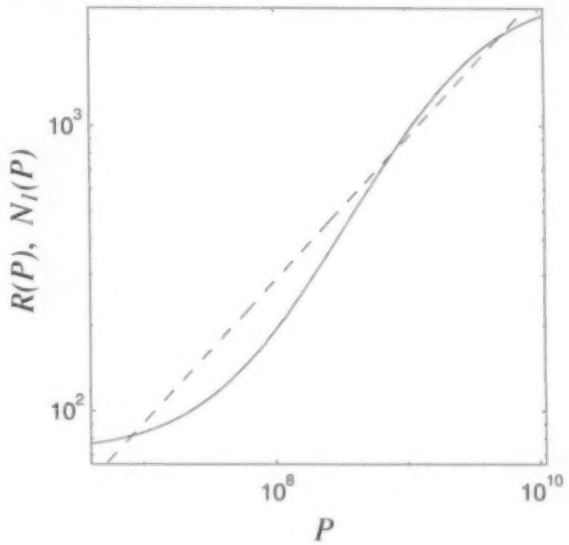
The dominant eigenvalue of a Leslie matrix is less than 1 if and only if the net reproductive number for the population is less than 1 (Cushing and Yicang 1994). Therefore, the dominant eigenvalue λ of the Jacobian matrix for this model is less than 1 if and only if

$$(B9)\quad 1 > \sum_{i=1}^{\omega}\frac{\partial R}{\partial N_i}\prod_{j=0}^{i-1}p_j = \left(\left(\sum_{i=1}^{\omega}\nu_i f_i \prod_{j=0}^{i-1}p_j\right)M + \left(\sum_{i=1}^{\omega}\mu_i m_i \prod_{j=0}^{i-1}p_j\right)F\right)R'$$

$$\Leftrightarrow 1 > 2F_0M_0N_1R'.$$

The stability analysis for Model 1 is completed by a graphical argument examining $R'(P)$ at each of the equilibria. Plotting $R(P)$ and $N_1 = (P/(F_0M_0))^{1/2}$ establishes that

Fig. B1. Graphical argument establishing stability of equilibria in eq. B9 for Region 2 in Fig. 1*b*. The broken curve $N_1(P)$ intersects the solid curve $R(P)$ from below for the first and third intersections and from above for the second intersection.



$$(B10)\quad R' < \frac{1}{2(F_0M_0P)^{1/2}} = \frac{1}{2F_0M_0N_1}$$

at a single equilibrium or at the smallest and largest equilibria when there are three intersections of these curves (Fig. B1). This implies stability. The required inequality is reversed for the middle equilibrium when there are three intersection points, which implies instability. A stability analysis for Model 2 would be more involved and is not included here. The close match of the two sets of simulations for all parameter values indicates that the stability properties are essentially unchanged (Figs. 2 and 3).

In the simulations, a and ξ were treated as independent random variables in each year, and no serial correlation was considered. Other parameters were fixed at the values described in Appendix A. Parameter a was sampled from an asymmetric β distribution so that $a \in [0,1]$, and in most years, a was extremely small. Parameter ξ was drawn from a lognormal distribution. Simulations for both Models 1 and 2 were performed using each a and ξ data set. Simulations were repeated with one variable held constant and the other allowed to fluctuate according to its probabilistic distribution to assess the relative contributions to observed dynamics from fluctuations in a and ξ. Mean values for the two distributions were assigned from a uniform grid that covered the region of parameter space shown in Fig. 1. Coefficients of variation for both a and ξ distributions were kept equal for consistency. For low-variance simulations, $CV = 1/\sqrt{3}$, and for high-variance simulations, $CV = 4$. Forty simulations were performed for each assigned mean value, and each ran for 1000 years. Sample paths are shown in Fig. 2. For each run, the population was initialised in its equilibrium age distribution, and initial values for N_1 were randomly assigned from a uniform distribution. The first 500 years of data were

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discarded to allow the dynamics to equilibrate. Around the bifurcations, simulations were repeated over a finer grid of mean a and ξ values. In this region of parameter space, 60 simulations were performed for each mean value, and each ran for 5000 years. Sample paths for these longer simulations are shown in Fig. 3.

Appendix B references

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Calculation of biomass trend, exploitation rate, and surplus production from survey and catch data

Ray Hilborn

Abstract: If survey data and historical catches are available, it is possible to estimate the history of surplus production from a fishery as long as the scalar between the survey and absolute biomass can be estimated. Alternative hypotheses about present stock size correspond to alternative hypotheses about the scalar, so that by specifying an estimate of the current stock size or exploitation rate, the entire trend of surplus production can be calculated. An example, using monkfish (*Lophius americanus*) in the eastern United States, is given that shows quite robust conclusions to alternative assumptions about current stock size, and the analysis suggests that the surplus production from monkfish has been the highest during the period of lowest stock size.

Résumé : Lorsqu'il existe des données d'inventaire et des informations sur les récoltes commerciales du passé, il est possible d'estimer l'historique de la production excédentaire, à la condition que l'on puisse déterminer le scalaire entre l'inventaire et la biomasse absolue. Faire des hypothèses de rechange concernant la taille du stock actuel correspond à faire des hypothèses sur le scalaire, si bien qu'en spécifiant une estimation de la taille du stock actuel ou de son taux d'exploitation, on peut calculer l'entière tendance de la production excédentaire. Un exemple basé sur la pêche commerciale de la Baudroie d'Amérique (*Lophius americanus*) de la côte est des États-Unis montre qu'on peut obtenir des conclusions très robustes pour des hypothèses diverses au sujet de l'importance de la taille du stock actuel. L'analyse laisse croire que la production excédentaire chez la baudroie a été maximale durant les périodes où la taille du stock a été le plus faible.

[Traduit par la Rédaction]

Introduction

Modern stock assessment methods rely on using all available data sources to understand the relationship between fishing mortality and long-term yield (Hilborn and Walters 1992; Quinn and Deriso 1999). Elaborate mathematical and statistical models are often employed that almost always make a range of assumptions about the constancy of the production relationship over time. When frequent surveys are available, it is possible to directly calculate the surplus production if the scaling factor between the surveys and absolute biomass is known (Schaefer 1954, 1957; Hilborn and Walters 1992). In this paper, I show that the surplus production relationship may be very robust to the value of this scaling factor and that very useful model-independent estimates of surplus production and other important management quantities can be estimated directly from survey data. I provide an example using the stocks of monkfish (*Lophius americanus*) in the eastern United States.

Materials and methods

If a survey index is proportional to biomass and catches in biomass are known, there exists a simple relationship between the catch, the population size, and the survey:

$$(1) \qquad B_t = \frac{C_t}{u_t}$$

where B_t is the population biomass at the beginning of time t, C_t is the catch in biomass during time t, and u_t is the exploitation rate at time t. If we specify u for any time, then we can calculate B, or if we specify B, we can calculate u. Both u and B could be estimated by a formal method such as virtual population analysis or statistical catch-at-age or biomass dynamics models or simply represent a hypothesis regarding the level of exploitation.

We can then calculate the relationship between the survey index and the biomass:

$$(2) \qquad q = \frac{I_t}{B_t}$$

where q is the scaling factor between the index (I) and the biomass (B). This assumes that I is measured at the beginning of time t.

Having determined q, we can then reconstruct the history of biomass and exploitation rate:

$$B_t = \frac{I_t}{q}$$

$$(3) \qquad u_t = \frac{C_t}{B_t}$$

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Table 1. Survey data from autumn NMFS cruises, and estimated live weight landed for northern and southern monkfish stocks (all data from NFSC 1997).

Year	Northern biomass index from autumn survey	Southern biomass index from autumn survey	Northern live weight landed (t)	Southern live weight landed (t)
1963	3.76	3.72		
1964	1.71	5.49	43	19
1965	2.51	5.16	37	17
1966	3.27	6.99	299	13
1967	1.28	1.12	538	7
1968	2.04	0.89	448	2
1969	3.70	1.14	256	4
1970	2.24	1.36	196	12
1971	2.91	0.79	212	10
1972	1.40	4.92	435	23
1973	3.11	1.99	707	136
1974	2.06	0.71	1 195	100
1975	1.71	20.40	1 853	279
1976	3.39	1.08	2 234	425
1977	5.57	1.87	3 134	827
1978	5.10	1.39	3 888	1 338
1979	5.13	2.28	4 014	3 370
1980	4.46	1.87	3 695	3 947
1981	1.98	2.86	3 217	2 274
1982	0.94	0.65	3 858	3 655
1983	1.62	2.15	3 848	4 084
1984	3.01	0.74	4 200	3 609
1985	1.44	1.32	4 615	4 104
1986	2.35	0.55	4 326	3 951
1987	0.87	0.27	4 957	3 705
1988	1.52	0.55	5 063	4 482
1989	1.38	0.63	6 378	8 290
1990	1.00	0.43	5 770	6 952
1991	1.24	0.78	5 690	9 382
1992	1.10	0.31	6 922	13 536
1993	1.04	0.29	10 182	14 880
1994	0.97	0.61	7 829	11 444
1995	1.71	0.39	12 772	7 835
1996	1.07	0.39		
1997	0.67	0.59		
1998	0.95	0.50		

Finally we can calculate the surplus production (S) for each year:

$$(4) \qquad S_t = B_{t+1} - B_t + C_t$$

This lets us examine the history of stock size, exploitation rate, and surplus production without making any assumptions except the exploitation rate in one year, the linear relationship between the index of biomass and real biomass, and the tautology given in eq. 4.

Results

Trawl surveys have been conduced by the National Marine Fisheries Service (NMFS) in the Atlantic during autumn since 1963 and during spring since 1968. The status of monkfish has been assessed using the length frequency in the autumn survey (NFSC 1997), and the stock is now considered depleted and overfished with a rebuilding plan proposed in 1999. The stock is divided into a northern and a southern stock and separate assessments are performed. Table 1 shows the autumn survey indices for the two stocks and the estimates of the tonnes live weight landed (NFSC 1997). Figure 1 shows the surplus production trend over time for the northern monkfish stock for New England if we assume that the stock size in 1996 was 60 000 t or an exploitation rate of approximately 15%. This is the estimated exploitation rate from the NFSC (1997). The index has been smoothed by calculating the 5-year running average. I have run simulations that show no impact of smoothing on general trends in surplus production, and issues regarding smoothing are discussed below.

The general pattern of calculations is very robust to estimates of 1996 stock size: only the axes change. Figure 2 shows the same relationship when the 1996 stock size is set to 30 000 t. This analysis has a single parameter, q, that relates biomass to the survey index, and rather than trying to estimate q from a model, we simply perform sensitivity analysis by

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Fig. 1. Trends in biomass (thick line) and exploitation rate (thin line) for the northern monkfish stock if the 1996 population size was 60 000 t; 5-year running average used.

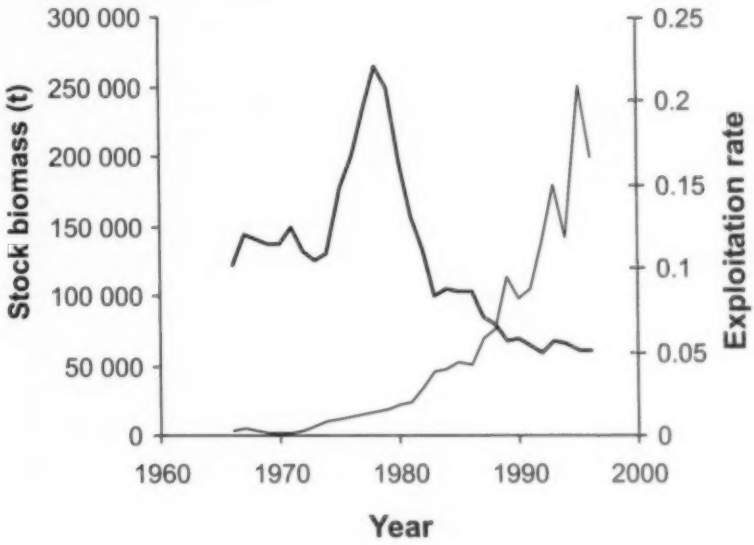
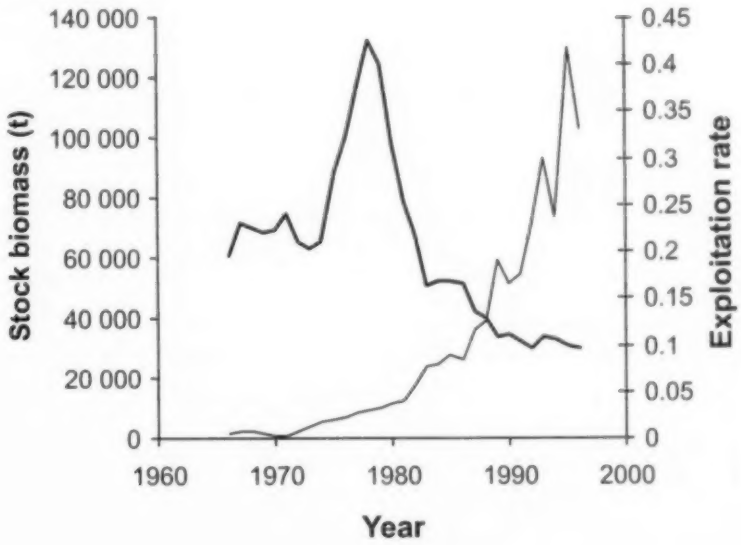


Fig. 2. Trends in biomass (thick line) and exploitation rate (thin line) for the northern monkfish stock if the 1996 population size was 30 000 t; 5-year running average used.



exploring the consequences of different biomass values, which, in turn, become different *q*s.

Figure 3 shows the trend in biomass and the estimated surplus production when the 1996 population size is set at 60 000 t. Note the strong rise in surplus production as the pulse in biomass comes through in the mid-1970s and then the strong negative surplus production as this biomass disappears. Also note that in the 1990s, a time of rising catch and low biomass, the surplus production remained high. We can then look at the trends in surplus production over time; in Table 2, I have averaged surplus production by decade (i.e., 1971–1980, 1981–1990, 1991–1998).

The key feature in Table 2 is that the surplus production in the 1990s was high because the index of biomass did not declined and large catches were taken. Surplus production in the 1970s is high because of the pulse in production in the late 1970s and it is low in the 1980s because of the decline in biomass. We can see that the estimated surplus production in the 1990s is reasonably insensitive to assumptions about absolute stock size, simply because the index did not change

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Table 2. Annual surplus production (t) estimated for different 1996 population sizes for the northern monkfish stock.

	1996 population size (t)		
Time period	30 000	60 000	90 000
1971–1980	5 093	8 077	11 060
1981–1990	–1 723	–8 098	–14 474
1991–1998	7 980	7 166	6 352

Table 3. Annual surplus production (t) estimated for different 1996 population sizes for the southern monkfish stock.

	1996 population size (t)		
Time period	20 000	30 000	60 000
1971–1980	932	907	829
1981–1990	–1 772	–5 046	–14 865
1991–1998	10 527	10 332	9 750

Fig. 3. Trends in biomass (thick line) and surplus production (thin line) for the northern monkfish stock if the 1996 biomass was 60 000 t; 5-year running average used.

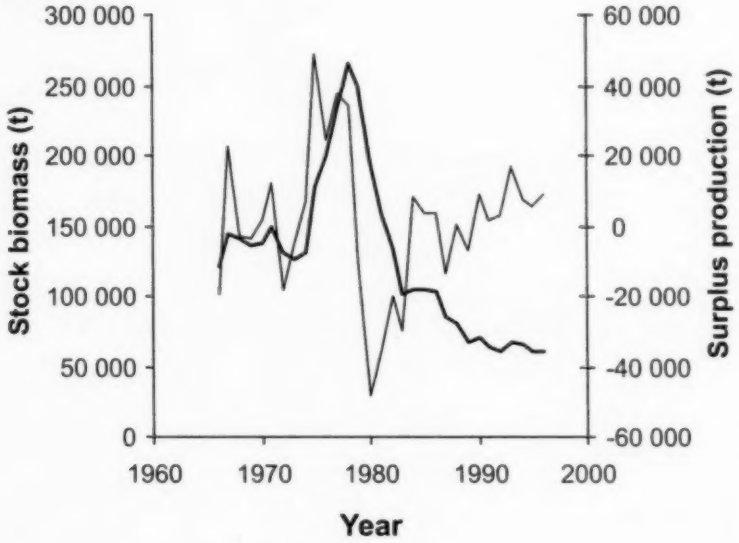

Fig. 4. Trends in biomass (thick line) and exploitation rate (thin line) for the southern monkfish stock if 1996 biomass was 30 000 t; 5-year running average used.

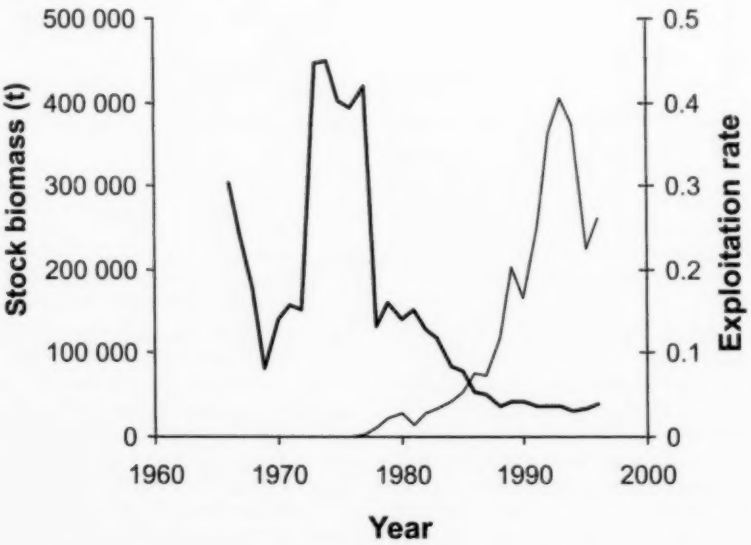

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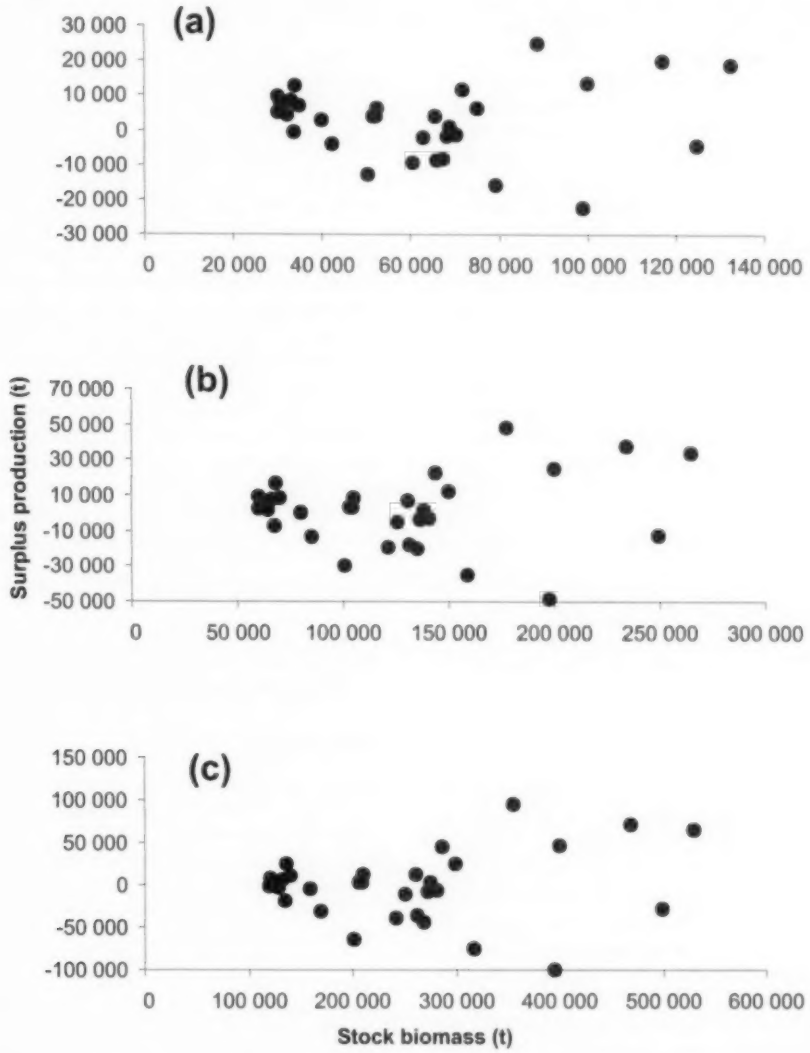
Fig. 5. Relationship between surplus production and biomass for the northern monkfish stock when the 1996 biomass is (*a*) 30 000, (*b*) 60 000, and (*c*)120 000 t.



much during this period, and therefore, surplus production equals catch. Also note that in all three scenarios, the average surplus production in the 1990s was well above the average combined for the 1970s and 1980s. For the southern stock of monkfish, we see in Fig. 4 a similar trend with a big pulse of biomass in the 1970s and a dramatic decline just before 1980. We can look at the trend in surplus production for the southern stock by decade in Table 3 where we see that there was almost no surplus production until the 1990s. Again, the estimated surplus production in the 1990s is insensitive to the estimated 1996 population size because the survey index did not changed much during the 1990s. Also note that we can easily account for any unreported catch simply by adding it to the surplus production for the decade where unreported catch might have occurred.

Traditional biomass dynamics methods (Hilborn and Walters 1992) would use similar data to relate a smooth function to the relationship between biomass and surplus production. Figure 5 shows the surplus production versus biomass relationship for the three 1996 biomass levels explored for the northern stock. There is no clear relationship, with large positive and negative surplus productions occurring at larger stock sizes, while there was consistent positive surplus production at lower stock sizes.

Discussion

The only two assumptions involved in this method of reconstructing surplus production are that the survey index is proportional to biomass (a standard assumption of all research surveys (Hilborn and Walters 1992)) and that the catch data record all removals. Thus, it provides a valuable tool for exploring the implied surplus production relationship whenever consistent indices of biomass data are avail-

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able. Neither of these assumptions may be strictly true for monkfish, and discussion of the failure of these assumptions illustrates the potential limitations of the method. There is a recognized but not estimated catch that occurred in earlier decades, particularly in the 1980s, largely in the form of unreported landings taken home for personal consumption. That may account for some of the negative surplus production estimated in that decade for both northern and southern stocks, although it is hard to imagine that it was several thousand tonnes. It is also possible, and suggested by fishers, that a significant fraction of the catch in the 1990s comes from areas outside the survey and that the survey does not index the entire stock biomass. If there is good mixing between the surveyed and not-surveyed areas, the survey index should be proportional to total biomass and the estimated surplus production should be correct. However, if the fish outside the survey area do not mix, a separate assessment of the areas that are newly fished would be needed. While I have used trawl survey indices, catch per unit effort data and even the estimated biomass from stock assessments could be used to reconstruct surplus production.

The failure of the survey to capture the entire population or the catch to reflect all removals will be equally, if not more, problematic in any other stock assessment method that uses catch and survey data. The survey data need not be continuous, as the surplus production can be calculated between surveys, regardless of the time between surveys. I recommend that any assessments that have an index of biomass available should calculate the surplus production using the method described in this paper in addition to any other stock assessment models being used.

Surplus production is a function of the somatic growth of individuals and new recruitment. As such, it may be misleading to plot the estimated surplus production against the current biomass, as the surplus production will depend on recruitments generated by earlier spawning stocks and body growth of recruits born earlier. Thus, to plot surplus production against stock size from an empirical analysis such as this is to assume equilibrium conditions, which clearly did not exist in monkfish and are generally rare in exploited fisheries. Nonetheless, it is clear that there have been great changes in surplus production in both northern and southern monkfish stocks that are unrelated to harvesting and, at a time when stocks are at low biomass, surplus production has been high.

I chose to use a 5-year running average of surveys to smooth the estimates of biomass and surplus production. Simulation testing showed no noticeable bias induced by smoothing, and the choice of smoothing interval had no impact on the conclusions reached for the monkfish data in this paper. I suggest that analysts explore different smoothing methods. The only bias found in the simulation trials was that the surplus production for any stock size is estimated to be higher than the equilibrium surplus production due to the recruitment lags mentioned above when fishing down a stock and to be lower than the equilibrium surplus production when rebuilding a stock.

For monkfish, this analysis shows that the surplus production in the 1990s was higher for monkfish in the south than in earlier decades, regardless of the assumption about the current stock size. For the north, the result is less clear, but if the current stock size is small, then, again, surplus production was the highest in the 1990s, a time of the lowest stock biomass. Thus, there is no evidence that the fishing has reduced the total production from monkfish. As this conclusion contrasts sharply with that reached by the NMFS assessment, the differences in the two views should be explored and possibly resolved.

Because long time series of survey data are available in many of the major commercial fisheries of the world, I recommend that agencies should calculate the surplus production using the method described in this paper in addition to any other stock assessment models being used.

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Fitness consequences of habitat use for juvenile cutthroat trout: energetic costs and benefits in pools and riffles

Jordan S. Rosenfeld and Shelly Boss

Abstract: To assess freshwater habitat requirements of juvenile anadromous cutthroat trout, *Oncorhynchus clarki*, we measured habitat preference and growth rates of young-of-the-year (YOY) and 1- to 2-year-old fish confined to either pools or riffles in Husdon Creek, British Columbia, during 1999. YOY preferred pools to riffles in habitat-preference experiments, despite normally occurring at lower densities in pools. YOY grew in both pools and riffles when experimentally confined to either habitat, but growth rates were higher in pools. Larger juvenile cutthroat trout, on average, grew in pools, but consistently lost weight in riffles, indicating that pools are a habitat preference for YOY but a requirement for larger fish. A bioenergetic cost–benefit analysis (based on swimming costs and energy intake from invertebrate drift) indicates that energetics alone are sufficient to account for avoidance of riffles by larger cutthroat trout, without having to invoke greater predation risk in shallow habitats. Energetics modeling demonstrates that the smaller size and energetic needs of YOY allow exploitation of habitats (e.g., pocket pools in riffles) that are unavailable to larger fish.

Résumé : Dans le but de déterminer les exigences d'habitat des jeunes Truites fardées anadromes, nous avons mesuré la préférence d'habitat et les taux de croissance chez des jeunes de l'année (yoy) et des poissons de 1–2 ans, confinés dans des profonds ou dans des rapides de Hudson Creek en Colombie-Britannique, Canada, en 1999. Les jeunes Truites fardées de l'année préfèrent les profonds aux rapides dans des épreuves de choix d'habitat, bien qu'elles soient normalement moins nombreuses dans les profonds; ces truites croissent dans les deux habitats lorsqu'on les confine expérimentalement dans l'un ou l'autre milieu, mais les taux de croissance sont supérieurs dans les profonds. En moyenne, les truites plus grandes croissent dans les profonds, mais elles subissent systématiquement une perte de masse dans les rapides, ce qui indique que les profonds sont un habitat préféré pour les jeunes de l'année, mais un habitat essentiel pour les truites plus grandes. Une analyse bioénergétique de coûts/bénéfices (basée sur les coûts de la nage et les apports d'énergie tirés de la dérive des invertébrés) montre que les considérations énergétiques seules suffisent à expliquer pourquoi les truites plus grandes évitent les rapides, sans qu'il soit nécessaire d'invoquer le risque accru de prédation dans les habitats peu profonds. La modélisation des relations énergétiques démontre que la taille et les besoins énergétiques réduits des truites de l'année leur permettent d'exploiter des habitats (e.g. les petites fosses dans les zones de rapides) qui ne sont pas disponibles aux poissons plus grands.

[Traduit par la Rédaction]

Introduction

Defining habitat requirements of fish is a necessary first step towards identifying and protecting critical habitat features. Habitat "requirements" is a commonly used but poorly defined expression; by habitat requirements, we refer to features of the environment that are necessary for the growth and persistence of individuals and populations. The habitat requirements of mobile life stages of fish are typically inferred from field surveys documenting the selection of different habitats (e.g., Moyle and Baltz 1985), in which selection is measured in terms of differential use or occupancy of particular habitats. However, it has been demonstrated that habitat use in the wild may not necessarily reflect habitat preference in the absence of biotic interactions, particularly for juvenile fish, which may be forced into suboptimal habitats by competition or predation (Werner and Hall 1988; Harvey 1991). This may lead to erroneous conclusions with respect to habitat preference (Mathur et al. 1985), including, for instance, the construction of biased habitat suitability curves for use in instream flow type methodologies. It is also implicitly assumed that habitat selection (differential habitat use in the wild) or preference (habitat selection in controlled choice experiments) accurately reflects the true benefits of using different habitats, but this assumption is rarely validated by measuring the fitness consequences (e.g., growth or survival) of using different habitat types (but see Lonzarich and Quinn (1995) for a good example of the survival consequences of differential habitat use).

Juvenile cutthroat trout (*Oncorhynchus clarki*) rear in very small streams that are vulnerable to habitat degradation from

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forestry and urbanization (Reeves et al. 1997), but their freshwater habitat requirements are poorly understood (Hall et al. 1997). Young-of-the-year (YOY) fish are most abundant in shallow riffle and glide habitat (Glova 1984), whereas larger parr are most abundant in deeper pool habitat (Rosenfeld et al. 2000). To determine whether habitat selection accurately reflects habitat preference, we performed habitat choice experiments with YOY in a natural stream. We then assessed the requirement for different habitats at summer low flow by measuring the growth rates of YOY (40–60 mm) and larger (120–160 mm) juvenile cutthroat trout confined to replicate pool and riffle habitats.

Preferential use of deeper pool habitat by fish is usually attributed to greater vulnerability to avian or terrestrial predators (Power 1984; Lonzarich and Quinn 1995). However, the costs and benefits of using shallow fast habitat versus deep slow habitat will also differ for drift-feeding fish, regardless of predation. Hughes and Dill (1990) developed a model for predicting microhabitat choice by drift-foraging Arctic grayling (*Thymallus arcticus*), based on the costs of swimming and the energy content of drifting invertebrate prey. We measured invertebrate drift rates in pools and riffles and used their model to estimate the energetic costs and benefits of exploiting pool versus riffle habitats for juvenile cutthroat trout. We then evaluated whether allometric differences in net energy gain were sufficient to account for observed differences in growth rates and habitat use by YOY and older fish, irrespective of predation risk.

Materials and methods

Experiments were performed in Husdon Creek, a small coastal British Columbia stream with an average bankfull width of 3.5 m and a summer base flow of approximately 30 $L \cdot s^{-1}$. Husdon Creek was chosen because it typifies the small-stream habitat in which juvenile anadromous coastal cutthroat trout are most abundant (Rosenfeld et al. 2000). The Husdon Creek basin is primarily second-growth forest, with a drainage area of 3.4 km^2. The fish community consists of juvenile coho salmon (*Oncorhynchus kisutch*) and cutthroat trout in three age-classes (YOY, 1+, and 2+ fish). Cutthroat trout and coho salmon densities are relatively high, averaging 0.92 and 0.2 fish/m^2, respectively, during summer base flow (J. Rosenfeld, unpublished data). The reach of stream used for experiments had approximately 75% canopy cover, a 1% gradient, substrate dominated by gravel and sand, and abundant large woody debris (LWD; 0.37 pieces of LWD/linear m) in a forced pool–riffle channel.

Enclosures were created by fencing off individual channel units, using 6.5 mm wire mesh supported by steel re-enforcing rod (the terms enclosure, channel unit, pool, or riffle are used interchangeably to refer to experimental units throughout the text). Fences were installed across the head and bottom of each pool or riffle, with the bottom skirt stapled to a wood two-by-four (1.625 × 3.625 in. (1 in. = 25.4 mm)) buried under the substrate. Substrate and cover within each channel unit were left intact. The 6.5-mm mesh was permeable to most invertebrates in the stream, with the exception of the largest instars of the stonefly *Pteronarcys* sp. Eight pools and seven riffles were isolated over a 400-m reach of stream, with five channel units as isolated enclosures and the remaining channel units in four separate groups of two to four adjacent channel units (adjacent channel units shared a common fence, with one channel unit upstream of the other). Channel units were selected on the basis of their obvious classification as pools or riffles (see Table 1 for differences in depth and velocity between habitats) and the additional criterion that fences could be installed without major habitat disturbance (e.g., removal of LWD). Channel units that met these criteria were scattered throughout the reach, and were similar in depth and surface area to pools and riffles surveyed over a 600-m length of stream that included the study reach. Although all enclosures had sufficient instream cover (cutbank, boulder, small or large woody debris) to provide refuge from terrestrial predation, cover in riffles was usually not associated with optimal foraging locations where fish could observe drifting invertebrates. To test whether cover affected the growth of large fish in riffles and pools, a 45 cm wide strip of 3-mm plastic screening attached to a wooden frame was placed across the stream 5–10 cm above the water surface in three riffles and three pools.

Because of the limited number of enclosures, growth rate experiments with YOY and older trout were run sequentially. All 15 enclosures were used to measure growth rates of larger juvenile cutthroat trout from 28 June to 3 August 1999, and eight enclosures were used to measure growth rates of YOY from 5 to 31 August 1999. The growth of larger fish was measured first because YOY were too small to be retained by the 6.5-mm mesh until the beginning of August. Stream temperature was similar in July and August, averaging 11.4 and 12.4°C, respectively. Fewer replicates were used for YOY growth because YOY were stocked at a higher density than larger trout and were therefore less subject to loss of replication through individual fish mortality (most enclosures were stocked with three to six YOY vs. a single larger trout).

Fish were removed from enclosures prior to stocking using minnow traps and multiple-pass electrofishing. Large fish stocked in enclosures were captured with minnow traps from the reach in which the experiments were performed. Large fish were stocked at a relatively low density (0.17 fish/m^2), to ensure that density-dependent effects did not confound habitat effects, and fish were stocked at equivalent densities in pools and riffles. Fewer large fish were stocked in pools than were captured in pools; however, large fish were not captured in riffles. Most enclosures had a relatively small surface area and were stocked with a single fish, but three of the larger pools received two fish and the largest pool received three fish. All fish were uniquely marked subcutaneously with visible implant elastomer (Northwest Marine Technology, Shaw Island, Wash.) to permit measurement of individual growth rates. Following marking, fish were held overnight to clear stomach contents, weighed to the nearest 0.01 g, measured to the nearest millimetre, and stocked in enclosures. Fish were collected 36 days later using minnow traps and electroshocking, and were again weighed and measured after being held overnight to clear gut contents.

Because many YOY were small enough to pass through a 6.5-mm mesh, a 3-mm mesh was placed over the original fencing. YOY were collected by electrofishing, because of low capture rates in minnow traps. YOY were individually marked, held overnight, weighed, measured, and released into four pools and four riffles (from 5 to 6 August) at the relatively low density of 0.5 fish/m^2 to prevent density-dependent effects. After 27 days, fish were recovered from enclosures by electrofishing, and were again weighed and measured after being held overnight. Screens were cleaned daily or as necessary throughout the experiments to maintain natural flow in enclosures.

Habitat preference of YOY

To assess the habitat preference of YOY, the center transverse fence separating adjacent pool and riffle channel units was removed to create three replicate pool–riffle sequences. The transverse wire-mesh fence separating each pool and riffle was replaced with a fine-mesh stop net buried beneath the substrate surface. We stocked individually marked fish at roughly equal densities (0.55 ± 0.13 fish/m^2 (mean ± SD)) in both pools and riffles on 8 August, leaving fish free to move between habitats within each replicate pool–riffle sequence. Fewer fish were stocked in riffles than in pools because riffles had smaller surface areas. Pools and riffles were isolated 1 week later when concealed observers on the stream

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Table 1. Physical characteristics of the pools and riffles used for growth experiments performed in Husdon Creek, B.C., in 1999.

Habitat	n	Area (m^2)	Mean depth (cm)	Maximum depth (cm)	Mean velocity ($cm \cdot s^{-1}$)
Pool	8	14.4 (8.0–22)	21.6 (16.7–25.4)	45.4 (37–51)	6.6(3.9–8.9)
Riffle	7	7.4 (5.3–9.1)	6.2 (5.0–7.7)	14.1 (12–18)	21.0 (15.6–24.5)

Note: Values are mean, with range given in parentheses.

bank quickly lifted buried stop nets by pulling on ropes attached to net ends. Fish were then recovered from separate pool and riffle habitats by multiple-pass electroshocking.

To examine the potential effect of predation by larger trout on YOY distribution, stop nets were buried again, and fish were returned to the channel unit from which they had been collected in each pool–riffle sequence. Large cutthroat trout (124–173 mm) were then stocked in pools at twice the density used in the growth-rate experiment. After 1 week, stop nets were again raised and fish were collected as in the first habitat preference experiment.

Measurement of foraging windows

Observations were made on cutthroat trout focal depths and velocities to estimate the size of foraging windows available to fish in pools and riffles; a foraging window is defined as the cross-sectional area of a stream surrounding a fish's focal point where it is capable of intersecting drift (Hughes and Dill 1990). A single focal point location of a drift-feeding fish was recorded in each enclosure for both small and large fish separately during the growth rate experiments (n = 8 pools and 6 riffles for large fish; n = 4 pools and 4 riffles for small fish). Observations were made from behind a portable blind or from higher up the stream bank using binoculars. When fish were using cover and could not be directly observed, focal location was estimated from trajectories of fish striking at twigs thrown onto the water upstream of the focal point. Only one large fish was observed holding in a riffle enclosure during the experiment. The most probable focal-point location of fish in the remaining riffles was estimated on the basis of close proximity to cover, relatively low current velocity, and least obstructed view upstream. The focal point of a single YOY was directly observed in each pool (n = 4) and riffle (n = 4) enclosure used for the YOY growth rate experiments. In all cases, fish were estimated to be holding at 60% of total water depth.

The general method of Hughes and Dill (1990) was used to estimate the size of the window of drift available to a fish upstream of its focal point. Stream depth and velocity were measured at 10-cm intervals along a transect through the focal point perpendicular to the current, and along a second transect 30 cm upstream of the focal point. These data were then used to estimate the volume of water and the total energy content of invertebrates drifting past a fish holding at the focal point (see below).

Measurement of invertebrate drift

Invertebrate drift was measured following the removal of larger fish at the end of the first set of growth experiments. A 250 µm mesh drift net (19 cm square mouth) was placed at the observed or most probable focal point of large fish in each pool (n = 8) or riffle (n = 7) enclosure. Drift nets were left for a minimum of 6 h between 10:00 and 18:00, with sets staggered over a period of 5 days because only four drift nets were available. Maximum depth and velocity at the center of the mouth of each net were measured at the beginning and end of each set. Velocities declined by an average of 2 $cm \cdot s^{-1}$ over the course of each set. Contents of drift nets were preserved in 5% formalin, and invertebrates were later sorted from detritus with a binocular microscope at 25× magnification and transferred to 80% isopropyl alcohol. Invertebrates were identified to genus using Merritt and Cummins (1984), with the exception of chironomids, which were identified to subfamily. Invertebrate length was measured to the nearest 0.05 mm, using a digitizing system and a binocular microscope equipped with a drawing tube (Roff and Hopcroft 1986).

Data analysis

Growth rate

Relative daily growth rate of individual cutthroat trout was calculated as final weight less initial weight divided by duration of the experiment and initial body weight. When multiple fish were present in an enclosure, growth rate was estimated as the average for all fish in that enclosure. Size (YOY vs. larger trout), habitat (pool vs. riffle), and cover (screen addition vs. no screen addition) effects on growth were analyzed as a three-way analysis of variance (ANOVA, n = 23), following log (x + 0.008) transformation to normalize data and remove correlation of variance with the mean. Effects on growth were re-analyzed as a two-way ANOVA, because one of the factors (screen addition) was not significant. The constant a in the log (x + a) transformation was selected on the basis of the criterion of maximizing the normality of regression residuals (Berry 1987), which was assessed using the Shapiro–Wilk statistic (SAS Institute Inc. 1989) and a frequency histogram of residuals. The assumption of homogeneity of variance in ANOVA was evaluated by testing for a significant correlation between the absolute value of residuals and predicted values. All analyses met assumptions of normality and homogeneity of variance at p = 0.05. Data analyses were performed using PC SAS (SAS Institute Inc. 1989).

To better describe the effect of habitat on cutthroat trout over a broad range of fish sizes (rather than as two size-classes), the relative growth rate for all individual fish (n = 45) was regressed against initial fork length. Although individual fish from the same enclosure are not independent and cannot be treated as separate observations without inflating probability values (cf. pseudoreplication), trout are territorial and growth rates of individual fish were somewhat independent insofar as they experienced different microenvironments within each enclosure. Relative growth rates were log (x + 0.007) transformed, to normalize variance and remove correlation of residuals with the mean. The log–log relationship of growth rate on fork length was back-transformed to its arithmetic power function to facilitate interpretation. To adjust for the inherent bias in back-transformation of logged relationships, a correction factor (CF) for scaling back-transformed logged values to arithmetic means was calculated as $CF = e^{(MSE/2)\times 2.303}$ (where MSE is mean square error) (Baskerville 1971; Sprugel 1983).

YOY habitat choice

Change in distribution between pools and riffles during habitat preference experiments (based on single trials with three replicates) was expressed as the change in initial and final densities in riffles (initial riffle density less final riffle density for each pool–riffle sequence, evaluated as a paired t test, n = 3) and as differences in final YOY densities between pool and riffle habitats (evaluated as a simple t test comparing final pool and riffle densities, n = 3).

Drift rate

Invertebrate biomass was estimated using published length–weight relationships (Meyer 1989; Sample et al. 1993; Benke et al. 1999). Drift concentrations, as biomass per metre cubed, were cal-

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culated by dividing the biomass of invertebrates collected during each drift set by the volume of water filtered by the drift net. Drift concentrations in pool and riffle habitats were then calculated as the mean for all drift sets in pools ($n = 8$) or riffles ($n = 7$). Separate drift concentrations were calculated for three size-classes of invertebrate (<2.5, 2.5–5.0, and >5.0 mm), as described by Hughes and Dill (1990), since reactive distance of drift-foraging fish depends on prey size. Differences in drift concentration between pool and riffle habitats were tested using a t test for each size-class. Drift concentrations in pools and riffles were combined to calculate an average concentration per size-class when there was no significant difference between habitats.

Estimation of energetic costs and benefits

Velocity and depth data collected along transects at fish focal points were used to estimate discharge (water volume per unit time crossing the lower transect) through adjacent 10 cm wide columns of water on either side of the focal point. Maximum lateral capture distances for the three size-classes of drifting prey were then used to estimate the volume of flow available to a fish striking prey from the focal point, as described in Hughes and Dill (1990). Three size-classes of prey were used because maximum capture distance (and therefore the volume of water scanned by a drift-foraging fish) depends on prey length (visibility). A more detailed description of the method for calculating the size of a drift-foraging window is presented in Hughes and Dill (1990).

Volume of water flowing through each 10 cm wide section of transect was calculated by multiplying cross-sectional area (CA) of the section by velocity (V). Total volume of water flowing through the drift window was then calculated by summing the discharge in each 10-cm section of transect within the reactive distance for each size-class of prey. Total energy content in the drift was calculated by multiplying total water volume and drift concentration (CONC) for each size-class of invertebrate. Invertebrate biomass was converted to energy content using a factor of 5200 Cal (= 21 790 J)·g^{-1} dry weight (Cummins and Wuycheck 1971). Energy gain was then calculated as 60% of energy intake, to adjust for energy loss due to specific dynamic action (15%), excretion (15%), and egestion (10%) (Hewett and Johnson 1992; Tucker and Rasmussen 1999):

$$(1)\qquad \text{energy gain} = \sum_{i=1}^{3}\sum_{j=-n}^{n} C_{adj}\cdot V_j \cdot \text{CONC 21 790 J}\cdot\text{g}^{-1}\cdot 0.6\cdot 3600\cdot 10^{-6}$$

where $i = 1,2,3$ represents each of the three size-classes of prey, $j = -n\ldots+n$ represents the number of sections to the left and right of the focal point, and 3600 and 10^{-6} are the respective factors to convert centimetres and seconds (the units of area and velocity, respectively) to metres and hours (see Hughes and Dill 1990).

Swimming costs (including basal metabolism) were calculated as a function of fish weight and focal velocity (V) using eq. 2 (Hughes and Dill 1990; adjusted to 10°C), assuming an average fish length and weight of 5.0 cm and 1.4 g, respectively, for YOY and 13.7 cm and 24.7 g, respectively, for larger juvenile trout:

$$(2)\qquad \text{swimming cost} = 10\cdot(C + M\cdot V)\cdot 19\cdot\text{weight}/1000$$

where C and M are dimensionless scalers ($C = 2.07 - 0.37\cdot\log(\text{length})$ and $M = 0.041 - 0.0196\cdot\log(\text{length})$ (Hughes and Dill 1990)).

Net energy gain was calculated as energy gain less swimming cost for each observed holding position for YOY and larger trout in pools and riffles. Relative energy gain was expressed as the ratio of net energy gain to swimming cost (energy gain divided by metabolic costs) to produce an index of growth potential for fish of different sizes. Relative energy gain was considered preferable to simple energy intake as an index of fish growth potential because it scales energy intake to metabolic costs. Relative energy gain (net energy intake/energy expenditure) was chosen over "efficiency" (gross energy intake/energy expenditure) because relative energy gain, like growth rate, can be negative; efficiency, on the other hand, can never be negative and is therefore a poor surrogate of growth potential for fish of different sizes.

Projections from energy modeling could not be normalized with data transformation, and habitat and size effects on energy gain, swimming cost, net energy gain, and relative energy gain were analyzed using a Wilcoxon rank sum (Mann–Whitney U) test evaluated with the Cochran–Mantel–Haenszel statistic (SAS Institute Inc. 1989), which is approximately χ^2 distributed. The ability of large fish to exploit riffle habitat was further evaluated by calculating net energy gain for large fish hypothetically foraging at observed focal points of small fish in riffle enclosures ($n = 4$).

Results

Growth rate

All large cutthroat trout were recovered from enclosures with no mortality over the course of the experiment; 33–100% of YOY were recovered from each enclosure (average of 67% in riffles and 82% in pools). These values probably overestimate mortality for YOY, since all stunned or killed YOY may not have been recovered in the complex natural stream habitat of enclosures despite multiple-pass electrofishing.

There was no significant effect of the addition of cover (screen) on growth rate of large cutthroat trout ($F_{1,12} = 1.2$, $p = 0.30$). There was a significant size ($F_{1,20} = 238.5$, $p < 0.0001$) and habitat ($F_{1,20} = 24.4$, $p < 0.0001$) effect on average relative growth rate within enclosures (Fig. 1). Growth rates of YOY were positive in both pool and riffle habitat, although growth was lower in riffles. In contrast, the growth rate of larger cutthroat trout was positive in pools but negative in riffles. Growth rates of individual fish showed a typical allometric decline in relative growth rate with increasing size (Fig. 2), with growth consistently higher in pools than in riffles across the entire range of fish length ($F_{1,44} = 42.6$, $P < 0.0001$). Relative growth rate was best described by back-transforming the log–log relationship (log(growth + 0.007) = 1.44 – 1.82(log length) + (0 for riffle, 0.29 for pool), $R^2 = 0.92$) to the corresponding power function: percent growth = ((27.5·length$^{-1.82}$·(1.94 for pool habitat, 1 for riffle habitat)) – 0.007)·1.02·100, where 1.02 is the correction factor for back-transformation and growth is in percent body weight per day.

YOY habitat choice

YOY demonstrated a clear preference for pool habitat. In the absence of predation risk or competition, there was a net movement out of riffles into pools (Table 2). The change in density in riffles was –0.42 ± 0.07 fish/m^2 (paired t test, $t_2 = -10.8$, $p = 0.009$), with the final density in pools (0.63 ± 0.09 fish/m^2) roughly three times that in riffles (0.23 ± 0.13 fish/m^2) ($t_2 = 4.47$, $p = 0.01$ for a significant difference in final density between pools and riffles). When larger cutthroat trout were introduced to pools in the second habitat-choice experiment, there was no significant effect on YOY distribution ($t_2 = 1.13$, $p = 0.32$ for a difference in final density between habitats). However, there was a net movement of fish into riffle habitat in the pool–riffle sequence with the least instream pool cover (Table 2), suggesting that the effect of

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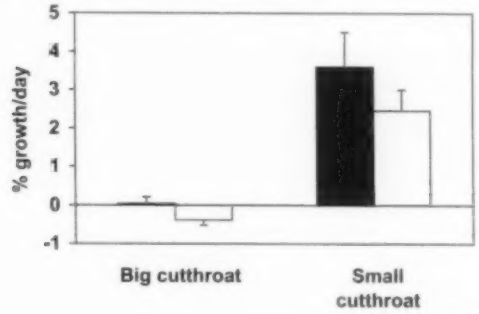
Fig. 1. Average growth rate (% body weight/day) of small and large juvenile cutthroat trout in pools (filled bars) and riffles (open bars).



predation on habitat choice is dependent on the availabilty of instream cover. When the buried stop net was raised in this sequence, one of the larger trout was observed trying to swim back into the pool from the riffle and regurgitated a marked YOY when recovered, demonstrating that predation risk for YOY was substantial.

Drift rates

Drift concentrations were significantly higher in riffles than in pools for the smallest size-class of drifting invertebrate ($t_{13} = 2.31$, $p = 0.038$), but were not significantly different between pools and riffles for the large and medium size-classes (Table 3).

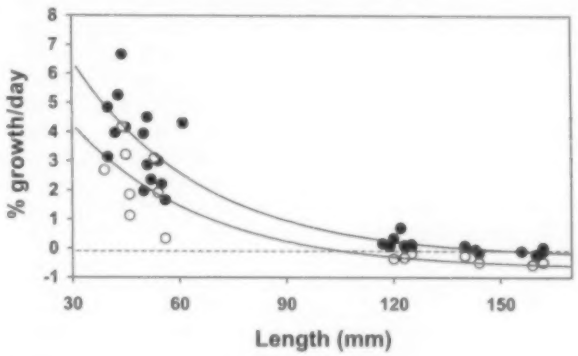
Energy costs and benefits

Observed focal velocities of YOY in pools and riffles were similar to those of larger juvenile cutthroat trout in pools, but estimated focal velocities for larger fish in riffles were higher and more variable (Table 4). Energy intake was significantly greater for large fish than for YOY (Fig. 3*a*) χ^2 (= 5.10, $p = 0.024$), and non-significantly higher in pools than in riffles χ^2 (= 2.72, $p = 0.10$). Swimming costs were significantly higher in riffles χ^2 (= 4.09, $p = 0.043$), although this was most pronounced for large fish (Fig. 3*b*). Net energy gain was higher in pools than in riffles χ^2 (= 11.68, $p = 0.001$) and positive for YOY in both habitats (Fig. 3*c*). Net energy gain was negative for larger trout in riffles (–71.0 ± 76.1 J·h^{-1} (mean ± SD)) but positive in pools. Energy gain relative to metabolic costs was significantly higher in YOY than in larger trout χ^2 (= 13.8, $p = 0.001$; Fig. 3*d*) and showed a pattern very similar to that of observed relative growth rates. Projected net energy intake for a large fish at the observed focal positions occupied by small fish in riffles was negative for three of four observations.

Discussion

Habitat-choice experiments with YOY cutthroat trout clearly indicate that habitat selection in the wild does not accurately reflect preference in the absence of predation and competition. YOY preferred deep pool habitat, despite occurring at highest densities in shallow habitats in field surveys (Glova 1984; Rosenfeld et al. 2000). However, preference for pool habitat does appear to accurately reflect the

Fig. 2. Growth rates (% body weight/day) of individual juvenile cutthroat trout in pool (filled circles) and riffle (open circles) habitats as a function of body length. The cluster of points to the left are young-of-the-year trout; the cluster to the right are larger juvenile trout. The upper line represents growth rate in riffles; the lower line represents growth rate in pools.



fitness consequences of habitat choice, since YOY growth rates were higher in pools than in riffles. Low densities of larger cutthroat trout in shallow habitats in the wild (Fausch and Northcote 1992; Rosenfeld et al. 2000) is also consistent with observed negative growth rates of larger cutthroat trout in riffles, and confirms that habitat selection by larger juveniles also reflects the fitness consequences of habitat use.

Differential growth rates of YOY and larger cutthroat trout in pools and riffles indicate different habitat requirements at summer low flow. Pools are a preferred habitat for YOY, but not a requirement, since they are capable of growth in riffles. In contrast, pools are a habitat requirement for larger juvenile cutthroat trout, which could not maintain a positive energy balance in riffles. Negative growth in riffles supports the paradigm that riparian management practices leading to a loss of pool habitat (e.g., through decreased LWD inputs; Murphy and Koski 1989) causes a decline in suitable rearing habitat for older fish. This emphasizes the importance of pools at a population level, since the freshwater rearing phase of anadromous cutthroat trout in small gravel–cobble streams is probably limited by the number of 1- to 2-year-old smolts rather than by YOY abundance (Fausch and Northcote 1992).

Although fish predation has been widely demonstrated to influence habitat use by juvenile fish (Gilliam and Fraser 1987; Werner and Hall 1988), the effect of introducing large cutthroat trout to pools in the habitat-preference experiment was ambiguous. While there was no effect of large trout on YOY density in the two pools with abundant cover, there was net movement out of pool habitat in the third pool with the least cover; although this observation is clearly in need of further replication, it suggests that predator effects on habitat choice are cover dependent. An alternative mechanism to cannibalism for displacement of YOY into riffles is competition from larger juvenile coho salmon that hatch earlier in the spring and also prefer pools (Glova 1986; Nickleson et al. 1992), particularly at high coho salmon densities or when instream cover is abundant. This is both a management and a conservation issue, since stocking juvenile coho salmon on top of native cutthroat trout stocks is common in coastal streams.

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Table 2. Number of young-of-the-year cutthroat trout at the start and end of habitat-choice experiments in the absence and presence of larger predatory trout for three replicate pool–riffle sequences.

	Initial no.		Final no.		Change in	Instream
	Pool	Riffle	Pool	Riffle	no. in riffle	cover (m^2)
No large fish	7	4	10	1	–3	2.5
	7	4	9	2	–2	2.7
	5	4	8	1	–2	0.8
Large fish added	10	1	10	1	0	2.5
	9	2	8	1	–1	2.7
	8	1	5	4	+3	0.8

Table 3. Densities ($mg \cdot m^{-3}$ ± SD) of drifting invertebrates in three size-classes in pool (n = 8) and riffle (n = 7) habitats, or both habitats combined (n = 15).

	Size-class		
	<2.5 mm	2.5–5.0 mm	>5.0 mm
Pool	0.180±0.008	0.011±0.013	0.028±0.075
Riffle	0.281±0.009	0.021±0.010	0.023±0.038
P	0.038*	0.14	0.89
Both habitats	0.023±0.010	0.016±0.012	0.026±0.058

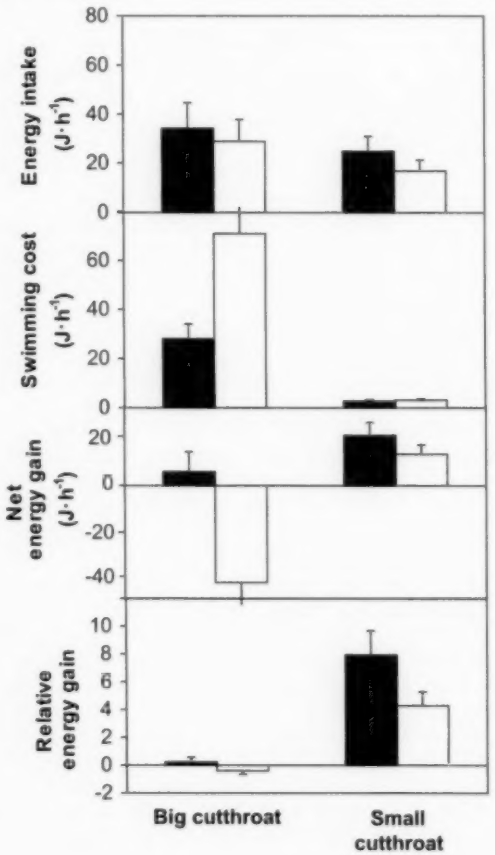
Note: P represents the probability of a significant difference between pool and riffle drift density for a given size-class; *, significant at $P < 0.05$.

Table 4. Velocities and total water depth at focal points of juvenile cutthroat trout in pools and riffles.

	Large fish		Young of the year	
	Pool	Riffle	Pool	Riffle
Total depth (cm)	17.2±4.4	6.3±1.7	16.5±3.8	6.0±1.3
Velocity ($cm \cdot s^{-1}$)	6.4±4.9	20.7±18.9	7.0±2.7	9.3±2.4

Note: Values are mean ± SD.

Fig. 3. Projected energy intake, swimming cost, net energy gain, and relative energy gain for small and large cutthroat trout in pool (filled bars) and riffle (open bars) habitats based on bioenergetic modeling.



The net outcome of coho salmon – cutthroat trout interactions is potentially complex (Polis et al. 1989) because coho salmon juveniles are both a competitor with YOY cutthroat trout and a prey item for older fish, but stocking coho salmon appears to contribute to long-term declines of cutthroat trout in coastal streams (Johnson et al. 1999).

Net energy intake from the bioenergetics model generated results consistent with the observed pattern of fish growth in pool and riffle habitats. Large fish were projected to lose weight in riffles, whereas small fish were projected to grow in both pools and riffles, with energy intake relative to metabolic costs 15–30 times higher for YOY. Although energetic models for drift-foraging fish have been used primarily to predict microhabitat choice rather than growth rate (Hughes and Dill 1990; Hughes 1992; Hill and Grossman 1993), the concordance of modeled and observed growth indicates their utility for predicting growth rates of drift-feeding salmonids (see also Hayes et al. 2000). Because the drift-foraging model of Hughes and Dill (1990) takes into account depth and velocity and is inherently three-dimensional, it also offers an alternative to modeling habitat capacity based on two-dimensional area-based measures of territory size (e.g., Grant et al. 1998). Two-dimensional territory-size models have proven useful for retrospectively explaining when density-dependent effects occur in streams, but implicitly assume that territory size is independent of habitat quality (e.g., pool vs. riffle habitat). Grant and Kramer (1990) found that predictions associated with a two-dimensional territory-size model do not apply well in deeper pool habitats, suggesting that estimates of territory size and habitat capacity that take volume and quality of habitat into account are likely to be more biologically realistic.

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Focal velocities of large fish in pools were partly based on strike trajectories rather than direct observation. This approximation of focal velocities is unlikely to have caused a serious bias in energetics calculations because of the relatively low variation in velocity and swimming costs within a pool, which would also have a minimal influence on the size of the foraging window. Because only one large fish was observed foraging in a riffle, focal velocities for estimating energetics in riffles were largely assumed using criteria of low velocity and proximity to cover. This was essentially a conservative assumption, since occupation of higher focal velocities would greatly increase swimming costs without appreciably increasing the size of the foraging window, leading to an even greater negative energy balance for larger trout in riffles. By selecting low-velocity focal points from the entire distribution of points in a riffle, we tested the extreme of the focal-point distribution most likely to give a positive energy gain for large fish in a riffle. Even at low focal velocities, the energetics model projected that fish would lose less energy by refuging than by foraging in most riffles, which is consistent with an absence of visibly foraging fish. In two of the riffles, the model predicted less energy loss by foraging than by refuging, which is consistent with the observation of a foraging fish (which lost weight) in one of these riffles.

Bioenergetic models used to estimate swimming cost are usually based on respiration rates of fish confined to swim tubes, in which flow is laminar and constant (Boisclair and Tang 1993), and may underestimate actual swimming costs of drift-feeding fish in turbulent flow (Daniel Boisclair, Université de Montréal, Department of Biological Sciences, Montreal, QC H3C 3J7, Canada, personal communication). Drift-feeding fish typically hold at a low-velocity focal point and strike at prey within a reactive radius that may carry them into higher-velocity locations. Fish striking from a focal point are likely to expend more energy leaving and returning to their focal point than predicted from a simple model based on holding at a single point in laminar flow. Despite the simplifying assumptions of present swimming-cost models, they appear to capture the average changes in swimming cost with current velocity and fish size, since the swimming-cost equation from Hughes and Dill (1990) (eq. 2 in Materials and methods) generates net energy gains consistent with the observed growth rates of cutthroat trout.

Selection of deeper pool habitat by larger fish is usually attributed to use of pools as refuges from avian or terrestrial predators (Power 1984; Lonzarich and Quinn 1995). Modeling net energy gain indicates that energetics alone is sufficient to account for the absence of larger juvenile cutthroat trout in shallow riffle habitats. We observed no mortality of larger juvenile cutthroat trout in either pools or riffles over the 30-day growth experiment, indicating little effect of terrestrial predators even in shallow habitats. In contrast, Lonzarich and Quinn (1995) showed that predation, most likely by herons, resulted in up to 50% mortality of larger juvenile steelhead trout in shallow pool habitat in small artificial channels adjacent to a larger stream in Washington. Lower mortality rates in Husdon Creek may reflect a lower abundance of avian predators in small forested streams, in contrast with higher densities of wading or diving birds in estuaries or riparian corridors adjacent to larger streams and rivers. Differences in mortality rates highlight the spatially variable nature of predation risk from terrestrial predators in small streams (e.g., Heggenes and Borgstrom 1988), which will influence survival and habitat selection to unknown degrees.

The habitat-specific growth model indicates that juvenile cutthroat trout need to shift from riffles to pools at a size of approximately 90 mm to maintain a net energy balance at summer base flow. This is analogous to the ontogenetic habitat shift of juvenile bluegill sunfish from the littoral zone of lakes to the more resource-rich but predator-abundant pelagic zone (Werner and Hall 1988). Werner and Hall (1988) attributed this size-dependent habitat shift to a trade-off between lower resource acquisition in the littoral zone versus greater predation risk from gape-limited predators in the pelagic zone. However, while the presence of YOY cutthroat trout in riffles represents a probable trade-off between lower resource acquisition in riffles versus greater predation risk in pools (or interspecific competition from coho salmon), the shift of larger cutthroat trout out of riffle habitat appears to be driven by a ceiling on maximum energy intake imposed by the habitat structure of shallow riffles rather than a trade-off between predation risk and resource acquisition.

Despite higher overall growth rates of cutthroat trout in pool enclosures, the largest trout marginally lost weight even in pools. By removing forage fish (YOY cutthroat trout and coho salmon) from enclosures prior to stocking, we forced larger cutthroat trout to feed exclusively on invertebrates. Larger juvenile cutthroat trout appear to be energy-limited by a diet of invertebrate drift even in pools, indicating the requirement for an ontogenetic dietary shift to facultative piscivory to maintain positive growth as they become larger. Despite a shift to piscivory, maximum energy gain in small-stream habitat probably remains limited by pool size and prey encounter rate, selecting for the final ontogenetic habitat shift of migration to the ocean where habitat structure no longer limits energy intake.

Holling (1992) hypothesized that habitat structure constrains resource acquisition so that discrete body-size ranges are optimal for exploiting resources at different spatial scales. The ability of smaller fish to exploit habitats that are energetically unsuitable to larger fish is consistent with this hypothesis, which may apply to many stream salmonids and some lentic species as well. YOY cutthroat trout effectively function to transfer energy up the food chain from unsuitable habitats to larger predatory trout in streams, although YOY also compete for drifting invertebrates to an unknown degree. Lower absolute energy requirements of smaller fish may also be a major adaptive force selecting for differences in maximum body size between species and provides a plausible explanation for the general decrease in average body size of fish along an upstream gradient (Hughes and Reynolds 1994). Although smaller fish have a greater metabolic requirement per unit biomass, they have lower absolute energy requirements, permitting persistence in habitats with insufficient energy to meet the demands of larger fish (e.g., small streams or shallow habitats in rivers).

Understanding the mechanisms underlying growth in different habitats is a useful first step towards defining both habitat requirements and adaptive constraints on habitat use. Human land use practices frequently alter the complex habi-

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tat structure that characterizes stream channels, thereby affecting the survival of both individuals and populations (Reeves et al. 1997). Logistic constraints often prevent the large-scale habitat perturbation experiments that are ideal for documenting the population-level effects of habitat change in streams (e.g., Hartman and Scrivener 1990). This study demonstrates an alternative way to infer population-level consequences of habitat change, by measuring the fitness consequences of using suboptimal habitats (e.g., riffles) that may become more abundant following disturbance.

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Quasi-cycles in crappie populations are forced by interactions among population characteristics and environment

Micheal S. Allen and Leandro E. Miranda

Abstract: Crappie (*Pomoxis* spp.) populations have been characterized as cyclic, with strong year-classes recurring at 2- to 4-year intervals. We evaluated the potential for cyclic trends in crappie populations using a population model that included a density-dependent stock recruitment function and random environmental variation. Slow, medium, and fast growth were simulated over 100 years. The model predicted highly variable recruitment that was strongly influenced by environmental fluctuation at low and intermediate stock densities. At high stock density, recruitment was low, even if environmental conditions were favorable. Significant quasi-cycles occurred, but they were not sustained throughout the time series due to random environmental fluctuation. Quasi-cycles occurred because intermediate stock density and favorable environmental conditions occasionally combined to produce a very strong year-class that greatly increased stock density in the following 1–3 years and produced low recruitment, even if environmental conditions were favorable. Empirical data from 32 years of sampling age-0 crappies at Ross Barnett Reservoir showed trends similar to the simulated fluctuations. We conclude that crappie populations likely do not exhibit true cycles but may show quasi-cycles as a result of the interaction between random fluctuations in environment and density-dependent mechanisms. The frequency of such quasi-cycles may be enhanced by rapid growth and high exploitation.

Résumé : Les populations de mariganes, *Pomoxis* spp., ont été définies comme cycliques, avec des classes d'âge plus importantes réapparaissant à des intervalles de 2-4 ans. Un modèle de population qui inclut une fonction de recrutement du stock dépendante de la densité et une variation aléatoire de l'environnement a permis d'évaluer l'existence de tendances cycliques chez les mariganes. Des croissances lentes, moyennes et rapides ont été simulées sur des périodes de 100 ans. Le modèle prédit, aux densités basses et intermédiaires de stock, un recrutement très variable qui est influencé par les fluctuations environnementales. Aux fortes densités, le recrutement est faible, même lorsque les conditions environnementales sont favorables. Il y a des quasi-cycles significatifs, mais ils ne se maintiennent pas au cours de séries temporelles à cause de la fluctuation aléatoire de l'environnement. Ces quasi-cycles apparaissent parce que la combinaison occasionnelle d'une densité moyenne du stock et des conditions favorables du milieu génère une classe d'âge beaucoup plus importante qui augmente la densité du stock pendant les 1–3 années subséquentes et donne lieu à un faible recrutement, même en présence conditions environnementales favorables. Des données empiriques provenant de 32 années d'échantillonnage de mariganes d'âge 0 au réservoir Ross Barnett montrent des tendances semblables aux fluctuations simulées par le modèle. En conclusion, les populations de mariganes ne subissent vraisemblablement pas de vrais cycles, mais elles affichent des quasi-cycles qui résultent de l'interaction entre les fluctuations aléatoires de l'environnement et les mécanismes dépendants de la densité. La fréquence de tels quasi-cycles peut être accrue par une croissance rapide et une forte exploitation.

[Traduit par la Rédaction]

Introduction

Some of the most intriguing examples of fluctuations in animal abundance are population cycles. Classic examples of cycles in abundance exist for mammals and bird populations (reviewed by Begon et al. 1996). Population cycles also occur in fish populations (Townsend 1989; Myers et al. 1998). Suggested mechanisms for cycles include delayed density-dependent interactions between recruits and stock fish (e.g., intraspecific competition for food, cannibalism) (Ricker 1997; Sanderson et al. 1999), chronic fluctuations in reproductive output based on age at first breeding by adults (Townsend 1989; Ricker 1997), and the interaction of stochastic processes with fishing mortality (Myers et al. 1998). Begon et al. (1996) noted that populations often do not show perfect cycles but may show a tendency toward cyclic population patterns.

Crappie (*Pomoxis* spp.) populations are often major components of fish assemblages in North American lakes and reservoirs. Population densities of crappies have been characterized as cyclic (Thompson 1941; Swingle and Swingle 1967), with strong year-classes recurring every 2–5 years.

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Crappie recruitment dynamics may be erratic with irregular circular patterns that lead to fisheries often characterized as "boom and bust" due to fluctuations in yield. Fluctuations have been attributed to density-dependent and density-independent factors. Several authors have reported that high fish density can reduce population fecundity and age-0 fish survival due to intraspecific competition (e.g., Swingle and Swingle 1967; Guy and Willis 1995) or cannibalism (Thompson 1941). Other authors have ascribed population fluctuations to variability in environmental conditions such as water level and water retention time (e.g., Guy and Willis 1995; Maceina and Stimpert 1998) and to variable angler exploitation among years (Webb and Ott 1991). We suspect that fluctuations are driven by the interaction within and among density-dependent and density-independent factors and that the unpredictability of the fluctuations is a function of the strength of the interactions, which vary over time and space (Allen and Miranda 1998).

Simulation models have been useful for evaluating population cycles (Townsend et al. 1990; Myers et al. 1998). Because long-term data (i.e., >30–50 years) from fish populations are rare, models allow for the investigation of potential long-term trends in population abundance emerging from the interaction of density-dependent and density-independent effects. We used a population model to simulate fluctuations in crappie populations. Our objective was to identify the mechanisms that might drive fluctuations in crappie population abundance.

Methods

We used an age-structured population model to simulate a white crappie (*Pomoxis annularis*) population. The age-structured model increased biomass of the population by age-specific somatic growth, reduced population abundance by age-specific natural, fishing, and hooking mortalities, and increased abundance by recruitment to age 1. The model simulated density-dependent responses of growth, natural mortality, and recruitment. Additionally, recruitment was modified by a random function that simulated environmental fluctuations. The model divided each year into three 4-month periods (February–May (period 1), June–September (period 2), October–January (period 3)) to simulate seasonal variations in population parameters. Below, we briefly describe the model (details are given in Allen and Miranda 1998) and the data used.

Growth

We modeled slow, medium, and fast growth representing 1 SD below the mean, the mean, and 1 SD above the mean growth of crappie in a data set of 65 lake-years compiled by Allen and Miranda (1995). Because seasonal growth increments for crappies were not available, we used estimates published for bluegill (*Lepomis macrochirus*) (Moyle and Cech 1988) and assigned 0.2 of annual growth to seasonal period 1, 0.5 to period 2, and 0.3 to period 3. Density-dependent growth was modeled as a decaying exponential function of stock density fit to empirical data from Ross Barnett Reservoir, Mississippi, described by Allen and Miranda (1998). The model assumed that all fish of a given age were of the same length and that there was no gender-specific growth rate, as none was reported in an extensive review conducted by Carlander (1977).

Initial population density and age structure

The population density and age structure used to initiate simulation experiments were derived by applying annual mortality to the average numerical density (34·ha^{-1}) of adult white crappies longer than 20 cm in Ross Barnett Reservoir (1963–1994). In this reservoir, white crappies usually reach 20 cm and enter the fishery by age 3 (T. Holman, Mississippi Department of Wildlife, Fisheries and Parks, P.O. Box 451, Jackson, MS 39205, U.S.A., unpublished data). Given 0.3 annual natural mortality and density of fish age 3 and older, we adjusted density to include age-2 fish (34/(1 – 0.3) = 49) and age-1 fish (49/(1 – 0.3) = 70) in the population density estimate. We distributed the 70 fish across ages 1–7 according to existing annual mortality rates.

A 10-year period of instability in population density and structure occurred when simulations began. This instability resulted from the stable age distribution's adjustment to the applied rate of recruitment and mortality. After this period of instability, the populations formed a new, stationary age distribution. Results exclude the 10-year instability period.

Recruitment function

Recruitment to age 1 was modeled with a dome-shaped curve identified by empirical data to best describe the relationship between density of crappie stocks and recruitment (Allen and Miranda 1998). The curve was characterized by (Ricker 1975)

$$(1) \qquad R = Se^{a(1-S/SR)}(1 - vd)$$

where R is recruits (number of age-1 fish), S is stock (number of fish older than age 1), a is the coefficient of density dependence determining the shape of the curve (a = 2.901), SR is the number of stock fish (older than age 1) when $S = R$ (SR = 70), v is mortality between samples taken at a fixed time period in the first year of life and the same period in the second year (v = 0.68, as per Allen and Miranda 1998), and d is a random deviate.

The effect that random environmental fluctuations may have on recruitment was evaluated by varying v through d. A random number generator produced normally distributed values of d with mean = 1 and SD = 0.6. We used 0.6 as the standard deviation because it emulated, both in magnitude of variation and in periodicity, recruitment dynamics of crappies in four reservoirs examined by Allen and Miranda (1998). Effects of environmental variation alone were evaluated by setting $a = 0$ in eq. 1 and effects of density-dependent recruitment alone by setting $d = 1$. Simulated populations that fluctuated due to random environmental conditions and those that fluctuated due to density-dependent recruitment were compared with those that fluctuated due to both.

Exploitation

We used the average exploitation rate (u = 0.42) for white crappies from Allen and Miranda (1995). Distribution of u among periods within each year was estimated from year-long harvest surveys made at Ross Barnett Reservoir (Allen and Miranda 1998). The surveys indicated that 58, 19, and 23% of the annual crappie harvest occurred during periods 1, 2, and 3, respectively. Given that 58% of the harvest occurred in period 1, an annual exploitation of 0.42 would result in $u_{\text{period 1}} = 1 - e^{0.58 \log_e(1-0.42)}$. Correspondingly, $u_{\text{period 2}}$ was estimated at 0.10 and $u_{\text{period 3}}$ at 0.12 for the mean u of 0.42. Exploitation was applied only to fish 20 cm and longer.

Natural mortality

Annual natural mortality estimates averaged about 0.3 for white crappie populations (Allen and Miranda 1995). No data were available concerning seasonal fluctuations in natural mortality. Therefore, natural mortality rates were assumed to be 0.11 in each of the three periods (i.e., $1 - e^{[\log_e(1-0.3)]/3}$). Density-dependent natural mortality was modeled as a rising exponential function of stock density fit to empirical data from Ross Barnett Reservoir and illustrated by Allen and Miranda (1998). The model described the proportion of average natural mortality achieved as a function of stock density. Random fluctuations in natural mortality of stock fish were

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incorporated by varying mortality with a normally distributed random deviate with 0.6 SD.

Hooking mortality

Hooking mortality of fish that had to be released because they were smaller than the length limit was estimated from the literature. Hubbard and Miranda (1989) found 3% hooking mortality when white crappies were caught from shallow water (1.2 m), but mortality rates as high as 67% occurred when fish were raised from 16 m to the surface. Muoneke (1992) reported 9% hooking mortality of white crappies caught out of a reservoir. We arbitrarily used 10% hooking mortality of released fish during each period for all simulations.

Analyses

We conducted 100-year simulations for each level of growth. The number of recruits to age 1 and adult abundance (number of age 2 and older fish in the population) were recorded for each year. To assess whether modeled recruitment dynamics were completely random or contained serial autocorrelations (e.g., cycles or patterns), we used the Ljung–Box test (Ljung and Box 1978) using procedure ARIMA (SAS Institute, Inc. 1996). If the Ljung–Box test was significant, we conducted simple time series analyses described by Potts et al. (1984) to identify patterns. Cyclic patterns should contain positive autocorrelations among peaks (e.g., years 4, 8, 12, etc.) and negative autocorrelations for troughs (i.e., years 2, 6, 10, etc.) (Potts et al. 1984). We constructed correlograms to assess patterns in recruitment (Potts et al. 1984). Autocorrelations at each lagged year were tested for significance ($P < 0.05$) using procedure ARIMA (SAS Institute, Inc. 1996).

We also tested whether selected environmental conditions tended to be cyclic and potentially impart cyclicity on fish populations. For this, we used temperature and precipitation data recorded annually during February–May of 1930–1992 at a weather station operated by the Mississippi Agricultural Experiment Station, Starkville, Miss. Temperature represented the mean of minimum values recorded daily during the February–May period and precipitation the total for each period. We chose temperature because it can influence factors such as time of spawning, length of growing season, and food availability and precipitation because it can influence discharge and lake levels and thereby the entire aquatic ecosystem. The Ljung–Box test was used to examine whether temperature and precipitation time series were completely random or contained serial autocorrelations.

Recruitment data from 32 years of sampling at Ross Barnett Reservoir (described in Allen and Miranda 1998) were used as reference to assess if the simulated populations resembled empirical data. The Ljung–Box test was applied to examine whether density of white crappies in the reservoir exhibited serial autocorrelations over the 32-year period. If so, the time series analysis described earlier was used to characterize patterns that were compared qualitatively with those of the simulated populations. The purpose of this analysis was not to identify whether the simulated population closely tracked the Ross Barnett empirical data but to verify that the simulations were realistic.

Results

Intensity of simulated recruitment to age 1 was influenced by the interaction of density-dependent and density-independent factors (Fig. 1). When environmental effects were held constant and recruitment was allowed to vary in response to density dependence, recruitment averaged 83 fish·ha^{-1} and ranged from 61 to 96 fish·ha^{-1}. Conversely, when environment was varied at random and the density-dependence function held constant, recruitment averaged 76 fish·ha^{-1} and ranged from 19 to 184 fish·ha^{-1}. When environmental fluctuation and density dependence were allowed to interact, recruitment averaged 87 fish·ha^{-1} and ranged from 0 to 257 fish·ha^{-1}. Recruitment was random when environment was varied at random (Ljung–Box tests, $P = 0.33$) and not random when density dependence but not environment ($P < 0.001$) or density dependence together with environment ($P < 0.001$) controlled fluctuation.

Stock density influenced the intensity of recruitment fluctuation (Fig. 2). Simulated recruitment was highly variable at low and intermediate stock densities (<100 adults·ha^{-1}) but was <50 recruits·ha^{-1} at stock densities >150 adults·ha^{-1} (Fig. 2). At low and intermediate stock densities, environmental variation caused highly variable recruitment, suggesting that although the potential for a strong year-class existed, environmental conditions determined the magnitude of recruitment. Conversely, at high stock densities, recruitment was low, even though environmental conditions were random (i.e., sometimes favorable).

Recruitment fluctuated greatly among years, with strong year-classes recurring every 2–10 years (Fig. 3). Moderate peaks in recruitment were usually followed by 1–2 years of reduced recruitment, whereas strong peaks in recruitment were usually followed by very low recruitment in the following 1–2 years (Fig. 3). This occurred because intermediate stock density and favorable environmental conditions coincided to produce a strong year-class, which greatly increased stock density in the following year and led to low recruitment through density-dependent processes, even if environmental conditions were favorable. Minor changes in recruitment did not greatly affect stock density in subsequent years, often resulting in less variable recruitment for 1–2 years.

Rate of growth also influenced dynamics of recruitment (Fig. 3). Recruitment was not random in models containing density-dependent recruitment for any level of growth (Ljung–Box tests, all $P < 0.001$) and contained autocorrelations. Correlograms for the slow-growth model exhibited a significant, negative autocorrelation with 1- and 2-year lagged values and then an irregular pattern of alternating positive and negative autocorrelations in 2- to 4-year intervals. Autocorrelations were not significant through the entire time series (Fig. 3) but were significant ($P < 0.05$) at lag years 1, 2, and 12 and marginally significant ($P < 0.10$) at lag years 4, 10, and 13. The medium growth showed a similar pattern of alternating irregular negative and positive autocorrelations at 2–4 years with significant correlations at lag years 1 and 12 and marginally significant correlations at lag years 8 and 11. Conversely, the fast-growth model showed regularly alternating negative and positive autocorrelations, suggesting significant 2-year cycles. However, the fast-growth correlogram was "damped," with autocorrelations becoming nonsignificant through time (Fig. 3).

Empirical recruitment data for 32 years at Ross Barnett Reservoir showed highly variable recruitment with apparent oscillations (Fig. 4). Recruitment was not random as evidenced by the significant ($P = 0.005$) Ljung–Box test. The correlogram exhibited a general trend of alternating positive and negative autocorrelations at 2- to 3-year intervals with significant positive autocorrelations at lag years 1, 5, and 6 (Fig. 4).

Environmental variation showed both random and nonrandom trends. Precipitation during February–May of 1930–

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Fig. 1. Simulated crappie recruits to age 1 for models containing both density-dependent recruitment and environmental variation, only environmental variation, and only density-dependent recruitment. All simulations are run with the medium growth rate.

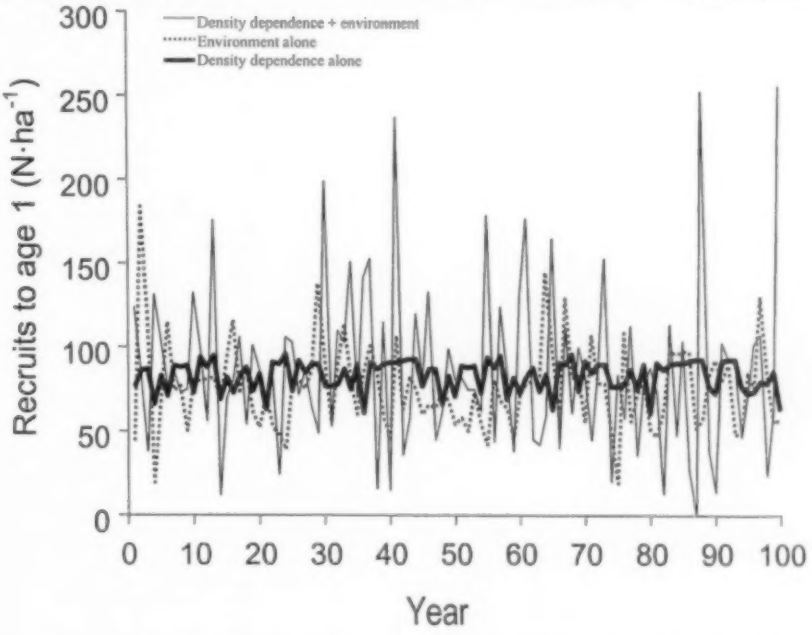

Fig. 2. Density of crappie recruits to age 1 plotted against density of adults (age 2 and older) for all simulations. Simulations with fast (circles), medium (squares), and slow (crosses) growth rates are shown.

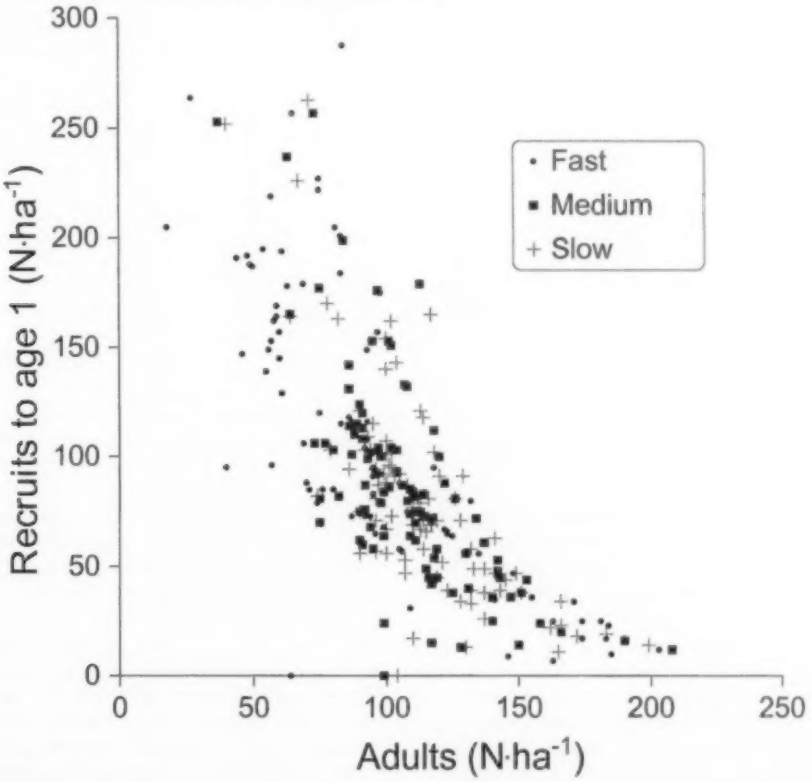

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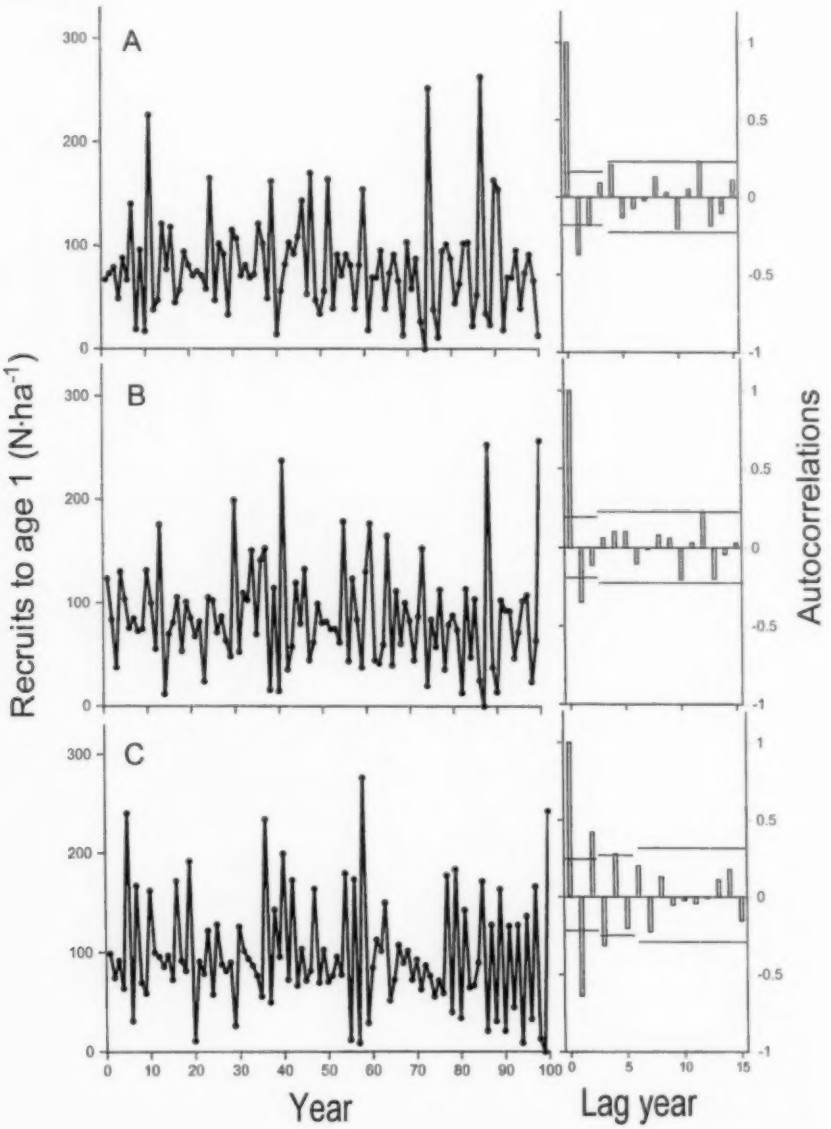
Fig. 3. Simulated time series of crappie recruits to age 1 for (A) slow, (B) medium, and (C) fast growth rate models. Panels to the right are autocorrelations in recruitment plotted against lag year for each simulation. Bars reaching the horizontal lines are significant autocorrelations ($P < 0.05$). Statistical power decreased with increasing lag year due to changes in sample size, resulting in changes in the position of the horizontal lines.



1992 did not differ from completely random (Ljung–Box test, $P = 0.92$). However, temperature patterns across the same time period were not random (Ljung–Box test, $P = 0.02$), but alternating positive and negative autocorrelations in temperature were not evident from a correlogram.

Discussion

Our model predictions at medium and slow growth appeared similar to recruitment dynamics at Ross Barnett Reservoir, with trends of 2–3 years of low recruitment usually followed by 2–3 years of strong recruitment. Growth rates at Ross Barnett Reservoir produced harvestable-size white crappies (20 cm total length) around age 3, similar to the medium growth rate that we modeled. Although our model exhibited recruitment dynamics that resembled those seen at Ross Barnett Reservoir, a perfect match was not expected because a number of factors not considered by our model also influence crappie dynamics. For example, environmental variation was assumed to be random. A test of this assumption showed that while rainfall appeared to be random during 1930–1992 at a station not far from Ross Barnett

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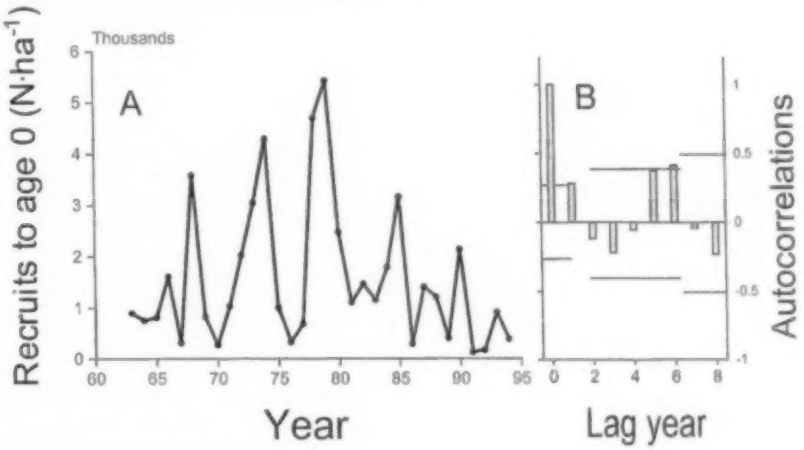
Fig. 4. Age-0 crappie (A) density over 30 years at Ross Barnett Reservoir, Mississippi, and (B) recruitment autocorrelations. Bars reaching the horizontal lines are significant autocorrelations ($P < 0.05$).



Reservoir, temperature was not random and could have contributed to shape patterns in white crappie population dynamics. Moreover, the model assumed constant exploitation over years, yet exploitation may also be cyclic where anglers abandon the fishery when catch rates decline and return after a strong year-class grows to maturity (Webb and Ott 1991). Conceivably, u has its own dynamics including lag periods where it exceeds average because interest in the fishery has not adjusted to the exhaustion of a strong year-class, or vice versa. Because no data were available to incorporate the dynamics of exploitation into our model, the model considered none of these potential interactions, yet it seemed to adequately sketch recruitment dynamics of white crappies in Ross Barnett Reservoir.

Truly cyclic populations have shown serial (i.e., repeated patterns) autocorrelations that are significant for up to 30 years (Begon et al. 1996). Data from our model and Ross Barnett Reservoir did not exhibit serial autocorrelations throughout the time series, suggesting that the actual and simulated crappie populations did not exhibit true cycles. Begon et al. (1996) termed such populations as "quasi-cyclic," where the time series exhibits a periodic tendency that does not persist, or tends to damp, due to disturbances caused by erratic environmental variation. Quasi-cycles resulted because years of very high recruitment increased stock density to produce low recruitment the following 1 year (fast growth) or 2–3 years (slow and medium growth), even if environmental conditions were favorable. The fast-growing white crappies showed significant 2-year quasi-cycles that became nonsignificant after a lag of 4 years, whereas the slow- and medium-growth populations exhibited quasi-cycles that, although they did not damp, were not serial (i.e., consistently significant throughout the time series). Damping was not evident because reduced growth protected a strong year-class from harvest and thereby helped stabilize stock abundance, yet fluctuations were not precisely serial because the next strong year-class had to await irregular periods for favorable environmental conditions. Two-year quasi-cycles under fast growth resulted because fish became harvestable at age 2 (20 cm total length), which quickly subjected a strong year-class to harvest, reduced stock abundance, and resulted in a high probability of strong year-classes at 2-year intervals. Thus, rapid growth could shorten the periods between peaks in recruitment and result in more frequent quasi-cycles.

Similar to our simulation of fast-growing white crappies, Cryer et al. (1986), Wyatt (1988), and Townsend and Perrow (1989) documented 2-year cycles in two roach (*Rutilus rutilus*) populations, but mechanisms for the cycles differed between populations (Townsend and Perrow 1989). In one lake, competition between age-0 and adult fish reduced growth and fecundity in years following good recruitment, resulting in high and low recruitment at 2-year intervals (Townsend and Perrow 1989). Age-0 and age-1 crappies are both zooplanktivorous (Mathur and Robbins 1971; O'Brien et al. 1984; Schael et al. 1991), and thus, production of a strong year-class could potentially affect food availability for the succeeding year-class. Density-dependent growth of crappies has been documented frequently for young (Swingle and Swingle 1967; Allen and Miranda 1998) and adult fish (Schramm et al. 1985; Miller et al. 1990). Occurrence of a strong crappie year-class could potentially reduce growth and survival of a subsequent year-class if food availability were limited, which could lead to 2-year cycles due to competition between successive year-classes. Our model used a Ricker-type stock–recruitment curve but did not explicitly model competition between successive age-classes. Conversely, in a second lake, roach exhibited rapid growth and matured in 2 years, resulting in strong recruitment at 2-year intervals (Townsend and Perrow 1989). Similarly, our model exhibited 2-year quasi-cycles at the fast growth rate, but quasi-cycles were due to stock fish being exposed to harvest early, which reduced stock density to trigger another quasi-cycle.

Sanderson et al. (1999) found that combined density-dependent effects resulted in 5-year cycles for a yellow perch (*Perca flavescens*) population. Following production of a strong year-class, competition between age-0 and age-1 fish and cannibalism on age-0 fish prevented recruitment for about 5 years until the strong year-class became sexually

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mature, when the cycle would repeat (Sanderson et al. 1999). Crappie population cycles due to cannibalism would likely result in cycles longer than 2 years because adults are not piscivorous until age 2 and older. However, cannibalism has seldom been documented as a regulatory factor in crappie populations (Thompson 1941), and our model did not consider cannibalism in determining recruitment.

Myers et al. (1998) found that high exploitation would intensify cycles in sockeye salmon (*Oncorhynchus nerka*) populations. Further simulations with our model also suggested that exploitation could influence cycles. Highly exploited crappie populations underwent rapid reductions in adult density, resulting in more frequent occurrence of quasi-cycles than simulations at low exploitation. However, periodicity of the cycles was not persistent throughout the time series, at high or low levels of exploitation. Random environmental fluctuations in our model apparently interacted with adult population density, and the influence of environment over density shifted periodically but randomly, forcing a damping in the autocorrelation.

Our model predicted that an interaction between stock density and environmental variation would combine to determine recruitment. Previous authors have attributed crappie recruitment fluctuations to independent effects of stock density (Swingle and Swingle 1967; Allen and Miranda 1998) and environment (e.g., McDonough and Buchanan 1991; Mitzner 1991; Maceina and Stimpert 1998). However, the lack of persistent cycles in simulated and empirical crappie populations underscores our contention that fluctuations are driven by both density-dependent and density-independent factors and that the unpredictability of the fluctuations is a function of the strength of the interactions, which vary over time. Density-dependent recruitment without environmental variation resulted in relatively minor recruitment fluctuations. Environmental variation greatly accentuated the occurrence and intensity of cycles, resulting in highly variable recruitment. We conclude that these cycles are likely not true cycles but instead are quasi-cycles resulting from the interaction between random fluctuations in environment and more predictable density-dependent mechanisms. The frequency of such quasi-cycles was influenced by growth rate and exploitation. Fishery managers should consider both environmental fluctuations and population density when addressing factors related to crappie recruitment dynamics.

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In situ studies with Asian clams (*Corbicula fluminea*) detect acid mine drainage and nutrient inputs in low-order streams

David J. Soucek, Travis S. Schmidt, and Donald S. Cherry

Abstract: In situ Asian clam (*Corbicula fluminea* [Müller]) studies may effectively mirror resident community responses to both acute toxicants and nutrient inputs in low-order streams. Clam survival and growth after 30 days in situ were compared with benthic macroinvertebrate community structural changes caused by acid mine drainage (AMD) and nutrient loading (measured as nitrate) in a small subwatershed of the North Fork Powell River, Virginia, U.S.A. Clam survival distinguished between two different levels of impact due to acidic, neutralized, and intermittent AMD inputs and was positively correlated with water column pH and negatively correlated with conductivity and metal concentrations. Survival was also positively correlated with relative abundance of the order Ephemeroptera, the most sensitive macroinvertebrate taxonomic group to AMD in this system. Clam growth was not related to AMD inputs but was positively correlated with nitrate concentrations and the relative abundance of the collector–filterer functional feeding group. These results suggest that transplanted clam studies accurately reflect benthic macroinvertebrate community responses to multiple stressors from point and nonpoint sources.

Résumé : Des études in situ sur la Mye asiatique (*Corbicula fluminea* [Müller]) peuvent représenter de façon réaliste les réactions de la communauté qui habite des ruisseaux d'ordre inférieur tant à des contaminations aiguës qu'à des apports de nutriments. La survie des myes et leur croissance après 30 jours in situ ont été comparées aux changements dans la structure de la communauté de macroinvertébrés benthiques causés par des rejets miniers acides (AMD) et des charges de nutriments (mesurées en nitrates) dans un petit sous-bassin hydrographique de la rivière North Fork Powell, en Virginie, aux U.S.A. Les données de survie des myes permettent de distinguer deux niveaux d'impacts causés par des apports d'AMD acides, neutralisés et intermittents; la survie est en corrélation positive avec le pH de la colonne d'eau et en corrélation négative avec la conductivité et les concentrations de métaux. La survie est aussi corrélée positivement avec l'abondance relative des Éphéméroptères, le groupe taxonomique de macroinvertébrés le plus sensible à l'AMD dans ce réseau. La croissance des myes n'est pas en corrélation avec les apports d'AMD, mais elle est directement reliée aux concentrations de nitrates et à l'abondance relative de la guilde alimentaire des collecteurs-filtreurs. Ces résultats laissent croire que des études sur des myes transplantées dans des cours d'eau reflètent de façon précise les réactions de la communauté de macroinvertébrés benthiques à des sources de stress multiples d'origine définie et diffuse.

[Traduit par la Rédaction]

Introduction

Complex combinations of environmental factors regulate aquatic community structure and population distributions (Merritt and Cummins 1996). Lotic benthic macroinvertebrate communities in particular are structured by biotic factors such as competition and predation and a number of abiotic factors, including climate, water quality, nutrient availability, stream order, and substrate composition. Studies of pollution impacts on aquatic communities are often inconclusive because a pollutant, such as an industrial effluent, may contain several interacting components. Some components may enhance an aspect of the community by providing a limited nutrient, while others have an inhibitory effect by causing toxicity (Wiederholm 1984). Ecosystems often receive inputs from multiple stressors, including both point and nonpoint sources, further confounding our understanding of community responses to pollutants.

In situ toxicity tests have gained popularity as replacements for, or supplements to, standard bioassessment tools such as laboratory toxicity testing and benthic macroinvertebrate sampling. They are thought to provide more environmental realism than laboratory tests by incorporating continuous exposure over an extended period of time to the multiple stressors that regulate indigenous communities and are less time and labor intensive than macroinvertebrate community sampling (Cherry 1996). The Asian clam *Corbicula fluminea* [Müller] is particularly useful as a biomonitoring tool because of its availability, 1- to 3-year life span, sedentary nature, sensitivity to different types of pollutants, and ability to accumulate organic pollutants and heavy metals (Doherty and Cherry 1988; Doherty 1990). Transplanted

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clams have been used widely to detect anthropogenic impacts in aquatic ecosystems, with endpoints ranging from survival and growth (Belanger 1991) to cellulolytic activity (Farris et al. 1988), valve movement (Allen et al. 1996), and DNA strand breakage (Black 1997).

While numerous studies have employed *Corbicula* as a biomonitoring tool, very few researchers (Farris et al. 1988) have compared transplanted Asian clam responses with those of benthic macroinvertebrate communities. The purpose of this study was to evaluate the correlation between transplanted Asian clam and indigenous community responses to acid mine drainage (AMD) and nutrient loading in first- to third-order streams. These objectives were accomplished by comparing the toxicological endpoints of clam survival and growth with benthic macroinvertebrate community indices, as community responses to both AMD and nutrient loading are well characterized (Wiederholm 1984; Hilsenhoff 1988; Kelly 1988). We hypothesized that (*i*) clam survival may be limited by acutely toxic AMD inputs in the upper portions of the watershed (first- to third-order streams) and (*ii*) clam growth is likely to be related to trophic status in low-order streams lacking AMD because they are filter feeders. Changes in those endpoints should be related to shifts in relative abundance of ecologically different groups of benthic macroinvertebrates. Therefore, clam growth and survival endpoints in low-order streams may provide different types of information to ecologists or risk assessors by responding in different ways to different types of pollutants.

Materials and methods

Sampling stations and groups

Puckett's Creek flows via Straight Creek and Stone Creek into the North Fork of the Powell River, Virginia, U.S.A., which contributes to the Tennessee River drainage (Fig. 1). Asian clam populations were well established in the Powell River before this study was conducted, so potential introduction of an exotic species was not a concern. Fifteen sampling stations were selected in Puckett's Creek and its tributaries, including Lick Branch, which received input from several AMD discharges. In addition, four stations were selected in higher-order (fourth to sixth) AMD-influenced streams of the same subwatershed of the North Fork of the Powell River. Stations were categorized based on level of AMD input as determined by location, stream order, and mean pH. Categorization of stations facilitated determination of clam sensitivity to the different types of AMD impacts relative to the benthic macroinvertebrate communities by providing treatments to compare using analysis of variance (ANOVA).

Five groups of stations were constructed as follows. The first group ($n = 5$) consisted of stations that were upstream of all known AMD inputs in Puckett's Creek and Lick Branch. Stream order for these five stations ranged from first to second. The second group ($n = 3$) consisted of stations ranging from first to third order and were subjected to intermittent AMD input. The intermittent designation was based on a wide pH range over time (e.g., 3.17–6.30 for station LB-5) or being downstream of such a station but upstream of any continuous AMD input (Soucek et al. 2000). The third group ($n = 4$) consisted of those stations in Lick Branch (first to second order) continuously subjected to AMD input and having acidic mean pH values (≤4.50). The fourth group ($n = 3$) consisted of stations in the lower, third-order section of Puckett's Creek below the confluence of Lick Branch; these stations were continuously subjected to AMD input but had circumneutral mean pH values (6.11–7.42) because of dilution. Therefore, group 4 stations will be referred to as neutralized AMD stations. The fifth group ($n = 4$) consisted of stations in Straight Creek, Stone Creek, and the North Fork of the Powell River. While all group five stations were downstream of various AMD inputs, they were differentiated from group 4 as having a higher level of dilution, being located in fourth- to sixth-order streams.

One additional station (SC-2) was selected in a third-order reach of the adjacent Straight Creek but was not used in the primary analyses because it was located in a different creek upstream of AMD input. This station was suspected of receiving diluted domestic input (possibly sewage) based on observations and nutrient measurements and therefore was used as a case study of clam growth in low-order streams where nutrient concentrations were elevated. Sampling at this station was conducted concurrently with, and was identical to, that for the other stations in terms of types of samples collected and analyzed.

Water column chemistry

To characterize stations according to the degree of AMD input and nutrient levels, water samples were collected at each station for analysis of selected water quality parameters on four occasions from fall 1997 to summer 1998. Either samples were brought to the laboratory, stored for 24 h at 4°C, and measurements taken under laboratory conditions or measurements were taken in the field. Sample pH was measured using an Accumet® (Fisher Scientific, Pittsburgh, Pa.) pH meter equipped with an Accumet® gel-filled combination electrode (accuracy <±0.05 pH unit at 25°C). Conductivity measurements were made using a Hach® (Hach, Loveland, Co.) conductivity/TDS meter. Dissolved oxygen, alkalinity, and hardness data for the watershed are provided in Soucek et al. (2000). In addition, water column samples were prepared for metals analysis according to standard methods (U.S. Environmental Protection Agency 1991). Samples were analyzed by inductively coupled plasma spectrometry for total aluminum (Al) and iron (Fe), which were previously determined to be the dominant metals in the system (Soucek et al. 2000). Lower detection limits were 0.001 and 0.002 $mg \cdot L^{-1}$ for Al and Fe, respectively. Water samples also were analyzed for nitrate (NO_3) according to standard methods (U.S. Environmental Protection Agency 1979).

Mean values for individual sampling stations were calculated for parameters of which more than one measurement was taken (i.e., pH and conductivity). Then, mean values for station groups 1–5 were compared by ANOVA. Student's *t* test was used for post hoc pairwise analysis at the $\alpha = 0.05$ significance level.

Benthic macroinvertebrate sampling

Benthic macroinvertebrate surveys were conducted according to the U.S. Environmental Protection Agency Rapid Bioassessment Protocols (Plafkin et al. 1989). Riffle, run, pool, and shoreline rooted areas were thoroughly sampled for 20 min per site using dip nets with 800-μm mesh. Two replicate samples were collected per site and mean values for all indices were calculated for each site. All organisms in each sample were identified to the lowest practical taxonomic level (usually genus) using standard keys (Pennak 1989; Merritt and Cummins 1996). Chironomids were identified as either subfamily Tanypodinae or non-Tanypodinae.

For community analyses, taxa were placed into six categories: Ephemeroptera, Plecoptera, Trichoptera, Hydropsychidae, Chironomidae, and "other." The "other" taxa category included the Megaloptera, Odonata, Coleoptera, nonchironomid Diptera, and noninsects. The Trichoptera group included hydropsychid caddisflies, but hydropsychids were placed into an additional category because they may reach high densities in response to dilute organic inputs or mild eutrophication (Wiederholm 1984). The relative abundance of each taxonomic group was calculated for each station by dividing, for example, the number of mayflies by the total num-

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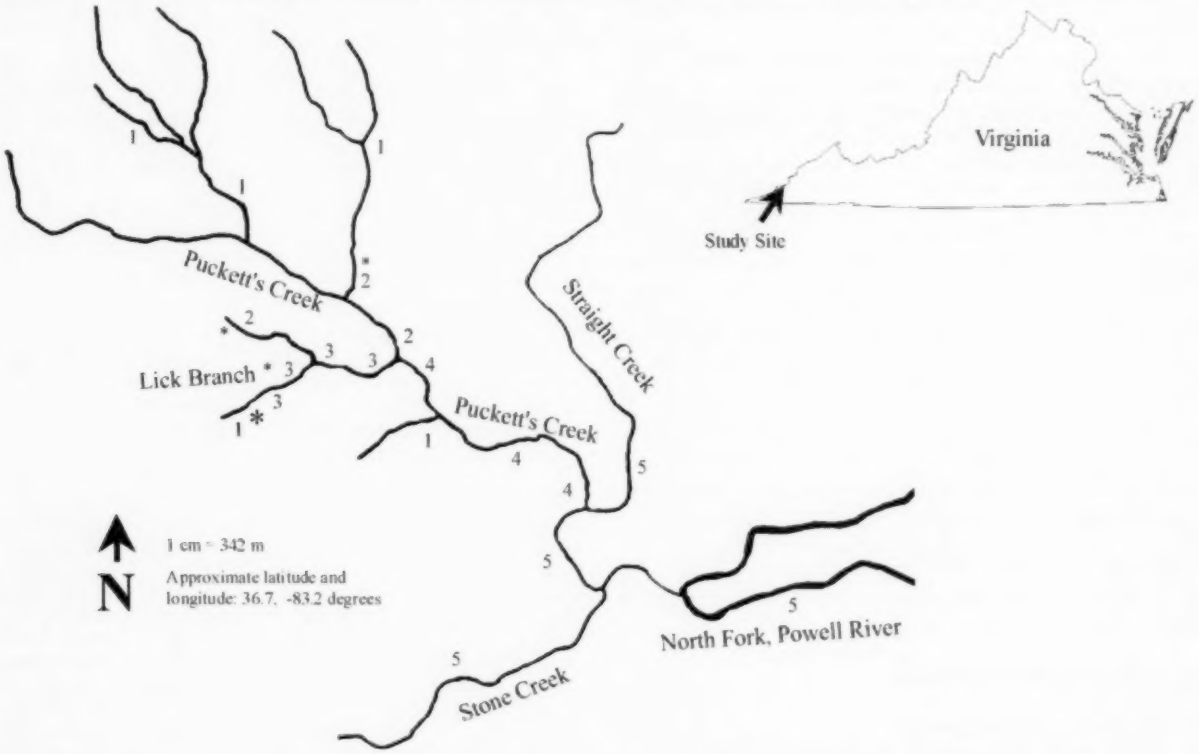
Fig. 1. Diagram of the Puckett's Creek watershed showing locations of sampling stations by group and AMD discharges. 1, upstream stations; 2, intermittent AMD stations; 3, acidic AMD stations; 4, neutralized AMD stations; 5, higher-order stations. The large asterisk indicates a continuously flowing AMD discharge, while the small asterisks indicate intermittent discharges.



ber of organisms. These values were compared within each station group by ANOVA and post hoc tests as described previously to determine which type of organisms were dominant within each station group.

Each taxon identified also was assigned to a functional feeding group as described in Merritt and Cummins (1996). Feeding groups included collector–gatherers, shredders, collector–filterers, scrapers, and predators. Tanypod chironomids were considered predators, and non-tanypod chironomids were collector–gatherers. Similar analyses were conducted as with the taxonomic groups, i.e., mean relative abundances of feeding groups were compared within station groups.

To further test for the potential presence of dilute organic input at station SC-2, family-level biotic index (FBI) values were calculated for the five upstream (group 1) stations and for station SC-2 (Hilsenhoff 1988). The FBI value gives an indication of the level of organic pollution from sources such as agricultural runoff or sewage based on tolerance values of arthropod families found at the site, with higher scores indicating more organic pollution.

In situ clam toxicity testing

For in situ toxicity tests, Asian clams were collected from the New River near Ripplemead, Va., using clam rakes. Clams were held in Living Streams® (Toledo, Ohio) at the Ecosystem Simulation Laboratory, Virginia Tech, Blacksburg, Va., until use. Individual clams were given one of five distinctive marks and measured for width to the nearest 0.01 mm using Vernier calipers prior to placement into bags for testing. Testing procedures consisted of tying five mesh bags, each bag containing five clams, to stakes at each sampling station. Bags were 18 cm wide by 36 cm long with a mesh size of about 0.5 cm^2. At the end of 30 days, clam bags were collected from each testing station and transported on ice to the laboratory. Clams were counted as dead or alive; clams found with valves separated, or that were easily opened, were considered dead. Surviving clams were measured again for width, and growth was calculated by subtracting beginning from ending width. Mean clam survival and growth values for station groups 1–5 were compared using ANOVA and pairwise analyses, as described previously, at the $\alpha = 0.05$ level.

Correlation analysis

To compare benthic macroinvertebrate sampling data with Asian clam toxicity test data for the watershed, bivariate correlation analyses were conducted. Both clam survival and growth were compared with taxonomic and feeding group relative abundances. Values from all 19 stations were included in the analysis for clam survival, but stations where all clams died were excluded from the correlation analyses for clam growth. Additional correlation analyses were conducted between clam survival/growth and water chemistry parameters to determine if the clam endpoints were related to AMD inputs or to nutrient levels at a given station. Significance of correlation was determined at the $\alpha = 0.05$ level.

Results

Water chemistry

The most extreme values for the four AMD-related parameters analyzed were observed at group 3 stations, where mean

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pH (3.71) was lowest and conductivity (1216 μmho·cm^{-1}), Al (30.7 mg·L^{-1}), and Fe (17.09 mg·L^{-1}) were highest (Table 1). The intermittent nature of AMD input at the group 2 stations was demonstrated by relatively high standard deviations about the means for pH, conductivity, Al, and Fe. The group 4 stations had a circumneutral average pH (6.84) but elevated values for mean conductivity (559 μmho·cm^{-1}) and Al (2.2 mg·L^{-1}). Mean water chemistry values for the group 5 stations were similar to those for the group 1 stations except for nominally elevated mean conductivity (469 μmho·cm^{-1}) and a significantly higher mean NO_3 concentration (0.40 mg·L^{-1}) than for any of the lower-order station groups (1–4).

Benthic macroinvertebrate communities

Benthic macroinvertebrate communities were sensitive to the various types of AMD input. At the group 1 stations, upstream of AMD input, Ephemeroptera and Plecoptera were the dominant taxa, making up 41.3 and 31.6%, respectively, of the mean total abundances (Table 2). The intermittent AMD stations (group 2) had similar assemblages in terms of which taxa were dominant, but mayflies (Ephemeroptera) were slightly less abundant than stoneflies (Plecoptera), chironomid numbers decreased, and relative abundance of the "other" group was not significantly different from that of the mayflies and stoneflies. At the acidic AMD stations, chironomids accounted for more than half of the organisms, while mayflies, stoneflies, and caddisflies made up less than 15% of the assemblages. The group 4 stations were dominated by the "other" taxa group, and at the group 5 or higher-order stations, no single group was dominant, as no significant differences were observed.

Changes also were observed in relative abundances of functional feeding groups in response to AMD. The collector–gatherer functional feeding group was dominant at all stations except for the neutralized AMD group (Table 2). At the group 1 stations, shredders were codominant, having a significantly higher relative abundance than the other three feeding groups. While shredders and collector–gatherers had high relative abundance values at the group 2 intermittent AMD stations, their values were not significantly higher than for the other groups. Collector–gatherers dominated the acidic AMD stations with a relative abundance of 67.5%. Predators were the dominant group at the neutralized AMD stations, making up almost half of the assemblages. At the higher-order group 5 stations, collector–gatherers and collector–filterers made up the greatest proportion of the macroinvertebrate assemblages, followed by predators, shredders, and scrapers.

In situ clam toxicity

The clam survival endpoint was sensitive to AMD inputs, differentiating between two levels of environmental impact. Average survival of clams at the end of the 30-day test was highest in groups 1 and 5 (92.8 and 92.0%, respectively), while none of the clams placed in the group 3 acidic AMD impacted stations survived (Table 3). As illustrated by standard deviations about means, survival at the stations with intermittent (group 2) and neutralized AMD input (group 4) was variable, with intermediate mean values of 54.6 and 36.0%, respectively. Groups 1 and 5 had significantly higher survival than group 2, which in turn had significantly higher survival than group 3 stations.

Clam growth was variable and less sensitive to AMD inputs when excluding all sites where 100% mortality of clams was observed (Table 3). While station groups consisting of first- to third-order reaches (groups 1, 2, and 4) had substantially lower growth (–0.08 to 0.06 mm) than the higher-order group 5 stations (0.37 mm), differences were not significant due to high variability.

Correlation analysis

Clam survival data compared with benthic macroinvertebrate indices produced three significant correlations (Table 4). Clam survival was negatively correlated with the relative abundance of chironomids and positively correlated with the relative abundance of mayflies (Ephemeroptera). The only functional feeding group that was correlated with clam survival was the scraper group, with a positive significant relationship.

Clam growth was negatively correlated with percent abundance of stoneflies (Plecoptera) but positively correlated with hydropsychid caddisflies and chironomids (Table 4). Growth also had a significant negative relationship with percent abundance of shredders and a positive relationship with collector–filterers.

Comparison of in situ toxicity test parameters with water chemistry data indicated that clam survival was significantly positively correlated with pH and negatively correlated with conductivity and Al and Fe concentrations in the water column (Table 5). NO_3 was not significantly correlated with clam survival; however, NO_3 had a significantly positive relationship with clam growth.

Clam responses to nutrient loading

Comparing nutrient levels and their effects in the absence of AMD, the upstream group 1 stations in Puckett's Creek had a mean NO_3 concentration of 0.16 ± 0.09 mg·L^{-1}, while station SC-2, a similar type of station in the adjacent Straight Creek watershed, had 0.56 mg NO_3·L^{-1}. Mean clam growth was substantially higher at station SC-2 (1.1 mm) compared with the mean value for the group 1 stations (0.062 ± 0.25 mm). Likewise, percent abundance of collector–filterers and FBI score were substantially higher in SC-2 (79.2% and 3.86, respectively) compared with the means for the Puckett's Creek group 1 stations (6.7 ± 6.4% and 2.54 ± 0.48, respectively).

Discussion

These results suggest that Asian clams may be used to detect two different types of pollution in headwater streams: acute toxicants and dilute nutrient inputs. In addition, in situ clam tests appear to accurately reflect benthic macroinvertebrate responses to these types of pollutants. Clam survival was sensitive to various levels of AMD inputs and was correlated with dominance of the most AMD-sensitive taxonomic groups (i.e., mayflies). Conversely, clam growth was correlated with dominance of benthic macroinvertebrate groups (i.e., hydropsychids and chironomids) that have been observed to thrive downstream of mild organic or nutrient inputs.

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Table 1. Mean (±SD) water chemistry values for station groups in the Puckett's Creek watershed.

Station group	pH	Conductivity ($\mu mho \cdot cm^{-1}$)	Al ($mg \cdot L^{-1}$)	Fe ($mg \cdot L^{-1}$)	NO_3 ($mg \cdot L^{-1}$)
1	7.27 ± 0.48 A	187 ± 99 A	0.2 ± 0.1 A	0.04 ± 0.02 A	0.16 ± 0.09 B
2	6.63 ± 1.35 A	525 ± 180 B	9.2 ± 10.8 A	4.32 ± 4.60 A	0.13 ± 0.04 B
3	3.71 ± 0.69 B	1216 ± 365 C	30.7 ± 13.4 B	17.09 ± 19.12 A	0.19 ± 0.07 B
4	6.84 ± 0.66 A	559 ± 6 B	2.2 ± 1.9 A	0.09 ± 0.07 A	0.14 ± 0.04 B
5	7.76 ± 0.30 A	469 ± 187 AB	0.19 ± 0.2 A	0.08 ± 0.11 A	0.40 ± 0.18 A

Note: Means in a vertical series followed by the same letter are not significantly different ($\alpha = 0.05$, ANOVA and Student's t test). Group: 1, no AMD impact ($n = 5$); 2, intermittent AMD ($n = 3$); 3, acidic AMD ($n = 4$); 4, neutral AMD ($n = 3$); 5, higher order ($n = 4$).

Table 2. Mean (±SD) percent composition by taxonomic and functional feeding group for the five station types in the Puckett's Creek watershed.

	Upstream group 1 ($n = 5$)	Intermittent group 2 ($n = 3$)	Acidic AMD group 3 ($n = 4$)	Neutralized AMD group 4 ($n = 3$)	Higher-order group 5 ($n = 4$)
Taxonomic group					
Plecoptera	31.6 ± 22.5 A	41.5 ± 19.1 A	10.9 ± 15.7 BC	25.8 ± 19.9 AB	9.9 ± 3.3 A
Ephemeroptera	41.3 ± 18.2 A	25.4 ± 14.4 AB	2.2 ± 3.6 C	1.4 ± 2.4 C	19.6 ± 14.3 A
Trichoptera	9.6 ± 6.3 B	9.9 ± 9.8 BC	0.4 ± 0.7 C	20.9 ± 6.0 B	18.7 ± 8.4 A
Hydropsychidae	6.4 ± 6.2 B	6.2 ± 8.8 BC	0.4 ± 0.7 C	13.2 ± 6.4 BC	16.6 ± 9.9 A
Chironomidae	6.5 ± 9.6 B	2.1 ± 2.5 C	59.4 ± 28.7 A	6.7 ± 5.9 BC	17.3 ± 9.2 A
Other	11.0 ± 12.6 B	20.9 ± 13.6 ABC	27.0 ± 15.7 B	44.8 ± 13.7 A	34.9 ± 18.2 A
Feeding group					
Collector–gatherers	42.9 ± 18.0 A	28.3 ± 12.9 A	67.5 ± 27.4 A	7.4 ± 2.5 BC	37.0 ± 12.7 A
Scrapers	8.6 ± 3.9 B	6.7 ± 3.5 A	1.5 ± 1.8 B	4.0 ± 5.3 C	8.6 ± 6.5 C
Collector–filterers	6.7 ± 6.4 B	7.0 ± 9.2 A	0.4 ± 0.8 B	15.4 ± 8.6 BC	23.2 ± 10.3 AB
Shredders	28.3 ± 22.5 A	40.0 ± 23.8 A	11.8 ± 17.0 B	26.8 ± 14.9 AB	11.7 ± 6.6 BC
Predators	8.5 ± 1.9 B	17.8 ± 10.6 A	18.6 ± 12.9 B	46.2 ± 16.4 A	19.3 ± 8.9 BC

Note: Means in a vertical series followed by the same letter are not significantly different ($\alpha = 0.05$, ANOVA and Student's t test).

Table 3. Mean (±SD) survival and growth in Asian clam in situ toxicity tests for station groups in the Puckett's Creek watershed.

Station group	Clam survival (%)	Clam growth (mm)
1. Upstream	92.8 ± 5.2 A	0.06 ± 0.25 A
2. Intermittent AMD	54.6 ± 47.7 B	–0.05 ± 0.04 A
3. Acidic AMD	0.0 ± 0 C	na
4. Neutralized AMD	36.0 ± 34.2 BC	–0.08 ± 0.03 A
5. Higher order	92.0 ± 5.6 A	0.37 ± 0.18 A

Note: Means in a vertical series followed by the same letter are not significantly different ($\alpha = 0.05$, ANOVA and Student's t test); na, not available.

Table 4. Correlation coefficients (r) between Asian clam in situ toxicity test endpoints and taxonomic and functional feeding groups of benthic macroinvertebrates in the Puckett's Creek watershed.

Comparison	r	p
Clam survival vs. Chironomidae	–0.526	0.0208
Clam survival vs. Ephemeroptera	+0.496	0.0304
Clam survival vs. scrapers	+0.469	0.0428
Clam growth vs. Plecoptera	–0.675	0.0113
Clam growth vs. Hydropsychidae	+0.650	0.0161
Clam growth vs. Chironomidae	+0.819	0.0006
Clam growth vs. shredders	–0.663	0.0135
Clam growth vs. collector–filterers	+0.745	0.0035

Note: All significant relationships ($p \leq 0.05$) are shown.

Clam survival effectively distinguished between different levels of environmental impact due to acidic, neutralized, and intermittent AMD inputs in this small watershed. While significant mortality of transplanted clams has been observed in response to organochlorine contamination (Hayward et al. 1996) and sewage treatment plant effluents (Belanger 1991), most transplant studies with Asian clams have investigated sublethal responses to pollutants such as DNA strand breakage (Black 1997), enzyme activity (Farris et al. 1988), bioaccumulation (e.g., Andrès et al. 1999; Gunther et al. 1999), and valve movement (Allen et al. 1996). These types of responses have been sensitive to low levels of pollution, but they may be too time intensive for a preliminary bioassessment of a whole watershed. In this study, the clam survival endpoint was sensitive to two different levels of environmental impact, and these survival responses were correlated with AMD-related water chemistry parameters such as pH, conductivity, and metal concentrations.

The benthic macroinvertebrate community responses to AMD in Puckett's Creek were similar to those observed in other AMD-impacted watersheds. For example, mayfly numbers often are depressed by mine drainages (e.g., Roback and Richardson 1969; Merrett et al. 1991; Nelson and Roline 1996). In Puckett's Creek, they were the most sensitive taxonomic group to AMD inputs, dominating the assemblages upstream of AMD inputs but comprising less than 2.5% of the assemblages at the acidic and neutralized AMD stations. Their relative abundance also was low at the stations with intermittent AMD inputs. Chironomid relative abundance

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Table 5. Correlation coefficients (r) between Asian clam in situ toxicity test endpoints and water quality data for the Puckett's Creek watershed.

Comparison	r	p
Clam survival vs. pH	+0.732	0.0008*
Clam survival vs. conductivity	–0.763	0.0004*
Clam survival vs. Al	–0.653	0.0045*
Clam survival vs. Fe	–0.535	0.0270*
Clam survival vs. NO_3	+0.337	0.1865
Clam growth vs. pH	+0.472	0.1423
Clam growth vs. conductivity	+0.205	0.5453
Clam growth vs. Al	–0.197	0.5617
Clam growth vs. Fe	+0.173	0.6106
Clam growth vs. NO_3	+0.647	0.0313*

Note: An asterisk indicates a significant relationship ($p \leq 0.05$).

also responded to AMD impacts, as has been observed in other studies (e.g., Roback and Richardson 1969; Armitage 1980; Rutherford and Mellow 1994), increasing in relative abundance at the acidic AMD stations compared with unimpacted sites. These findings suggest that this watershed was a good system to use in a comparison of transplanted Asian clam responses to AMD with those of the resident community.

To our knowledge, one other study has compared transplanted Asian clam responses with indigenous community responses (Farris et al. 1988). In that study, clam cellulolytic activity was related to the presence of suspended particles with bound metals, while invertebrate communities were most severely impacted where dissolved metals were highest. Clam and macroinvertebrate community endpoints were not compared statistically. In the present study, clam survival was positively correlated with relative abundance of Ephemeroptera and negatively correlated with Chironomidae. These were the most and least sensitive taxonomic groups, respectively, to various levels of AMD input. Thus, clam survival in transplant tests appears to mirror benthic macroinvertebrate community responses to AMD in this watershed.

Clam growth could not be determined at the most severely impacted sites (group 3) because all clams died, and at stations where clams survived, growth was minimal except in the fourth- to sixth-order stations (group 5). Furthermore, clams decreased in size at the group 2 and 4 stations, as has been observed in response to low levels of copper, zinc, and chrysotile asbestos (Belanger et al. 1986*a*, 1986*b*, 1990), but those means were not significantly different from the mean for the upstream stations due to variability within groups.

Although clam growth was not statistically sensitive to AMD inputs and was not significantly correlated with AMD-related chemistry parameters, it was positively correlated with NO_3 concentrations, suggesting that the nutrient loads explained variations in clam growth in this headwater system. Growth was greatest at the larger group 5 stations, which had the highest NO_3 concentrations and the greatest flow, being fourth- to sixth-order streams. Clam growth was negatively correlated with relative abundance of the Plecoptera taxonomic group and the shredder functional feeding group and positively correlated with relative abundance of hydropsychids, chironomids, and collector–filterers, providing further evidence of this nutrient-level connection with clam growth.

As filter feeders, the success of Asian clams in terms of building biomass is dependent on suspended and (or) dissolved carbon as a food source. Coarse particulate organic matter is generally the major carbon source in a small system of first- to third-order streams. Thus, shredders, which feed upon coarse particulate organic matter, should be one of the dominant functional feeding groups in low-order streams (Vannote et al. 1980), and they had the second highest relative abundance at the group 1 stations of this study. Collector–filterers generally are not as abundant as shredders in headwater streams because of the relative lack of fine particulate or dissolved organic matter. Therefore, the poor clam growth at the group 1 stations is not unusual; however, these results have other bioassessment implications.

Based on these data, one should expect poor growth of Asian clams at stations upstream of acutely toxic inputs in headwater systems, as was observed for the group 1 stations, unless some artificial nutrient source is available. Both hydropsychids (collector–filterers) and chironomids (collector–gatherers) have been observed to reach high densities in response to dilute eutrophication or organic input (Wiederholm 1984). Clam growth in this watershed was significantly positively correlated with the relative abundance of both hydropsychids and chironomids. Therefore, robust growth of clams in a first- to third-order stream may also be an indicator of dilute anthropogenic organic or nutrient inputs (i.e., dilute sewage or agricultural runoff).

To test this hypothesis, we conducted the comparison of clam growth and macroinvertebrate community indices for the Puckett's Creek group 1 stations versus the values for station SC-2, which was suspected of receiving elevated nutrient inputs. The NO_3 concentration at station SC-2 was 3.5-fold higher than the mean for the Puckett's Creek stations, and FBI scores and percent collector–filterer values responded accordingly with 1.5- and 11.8-fold increases, respectively. An FBI score of 2.54 (the average for Puckett's Creek group 1 stations) corresponds to an "excellent" rating with "organic pollution unlikely," while a score of 3.86 (station SC-2) indicates "possible slight organic pollution" (Hilsenhoff 1988). Likewise, clam growth was about 18-fold greater at station SC-2 than the mean for the Puckett's Creek upstream stations. Thus, clam growth in transplant toxicity tests appears to be related to nutrient levels and accurately reflects benthic macroinvertebrate responses to nutrient loading.

While clam survival and growth may provide much information about a small watershed, the tests are simple to conduct and cost little in terms of time and materials. Disadvantages of transplant studies with Asian clams include potential vandalism of test containers, predation upon test organisms, and potential release of propagules into previously uncolonized areas (Cherry 1996). However, these data suggest that transplanted clams may be useful tools for preliminary reconnaissance of small headwater systems that Asian clams have already colonized.

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Spatial and temporal variability in water temperatures in the littoral zone of a multibasin lake

Kerri P. Finlay, Hélène Cyr, and Brian J. Shuter

Abstract: Current models of the offshore thermal structure in lakes suggest that the thermal regime inshore should vary seasonally, and should vary systematically from upwind to downwind littoral sites. To test these hypotheses, we measured mean temperature and short-term (<2 days) temporal variability in water temperature at 23 shallow littoral sites in four basins of Lake Opeongo, Ontario, Canada. Water temperature in the littoral zone was most variable, both spatially and temporally, in the spring. Spring water temperature differed by as much as 7–15°C among littoral sites in different basins (median difference among sites = 2–4°C). Downwind sites were generally warmer and had more constant temperature than upwind sites, but the magnitude of these differences varied among basins of different sizes and shapes. Simple calculations of growth rates and of fish hatching times suggest that the observed variability in water temperature would result in differences in the productivity of different portions of the littoral zone.

Résumé : Les modèles actuels qui expliquent la structure thermique des eaux au centre des lacs laissent croire que le régime thermique près des berges doit varier d'une saison à l'autre et qu'il doit se modifier de façon systématique depuis les sites littoraux au vent jusqu'aux sites sous le vent. Pour éprouver ces hypothèses, nous avons mesuré la température moyenne et la variabilité temporelle à court terme (<2 jours) de la température de l'eau à 23 sites littoraux peu profonds dans 4 bassins du lac Opeongo, Ontario, Canada. La température de l'eau du littoral est le plus variable au printemps, tant dans l'espace que dans le temps. Nous avons observé des différences de température d'un site à l'autre allant jusqu'à 7–15°C selon les bassins (l'écart médian entre les sites = 2–4°C). Les sites sous le vent sont généralement plus chauds et ont des températures plus constantes que les sites au vent, bien que l'importance de ces écarts varie dans les bassins de tailles et de formes différentes. Des calculs simples des taux de croissance des poissons et des temps d'éclosion de leurs oeufs laissent présumer que la variabilité observée dans la température peut générer des différences de productivité dans les diverses sections de la zone littorale.

[Traduit par la Rédaction]

Introduction

Water temperature is affected by heat exchanges with the atmosphere and by physical mixing of the water column, usually by wind action (Imberger 1994). Several mechanistic and empirical models have been developed to predict water temperature and the thermal structure of lakes offshore (e.g., Spigel and Imberger 1980; Shuter et al. 1983; Fee et al. 1996). Such models indirectly suggest how water temperature should vary inshore, but these predictions are rarely tested (but see Matuszek and Shuter 1996). From a biological standpoint, the littoral zone of lakes is the most productive portion of aquatic ecosystems (per unit area; Wetzel 1990) and could be greatly affected by temperature fluctuations. Water temperature is a fundamental aspect of littoral habitat.

Under calm conditions, heat exchanges with the atmosphere determine the water temperature and circulation patterns in the littoral zone of lakes and reservoirs (Horsch and Stefan 1988; Monismith et al. 1990; James and Barko 1991). Heat fluxes across the water surface (cooling or warming) vary, diurnally and seasonally, in an irregular fashion, depending on local meteorological conditions (e.g., air temperature, cloud cover). It is well known that such heat exchanges at the surface result in larger changes in water temperature at shallower sites (Horsch and Stefan 1988; Monismith et al. 1990; James and Barko 1999). This differential heating–cooling creates an inshore–offshore temperature gradient that varies diurnally and seasonally, with warmer waters inshore when the atmosphere is warmer than the water (i.e., during the day, in the spring) and cooler waters inshore when the atmosphere is cooler than the water (i.e., at night, in the fall). In the Great Lakes, these temperature differences result in the well-known thermal bars, which effectively isolate inshore from offshore waters for several weeks each year (Ullman et al. 1998). In smaller lakes, the more subtle diurnal variations in inshore–offshore temperature gradients have been shown to trigger convective water exchanges between the littoral zone and the

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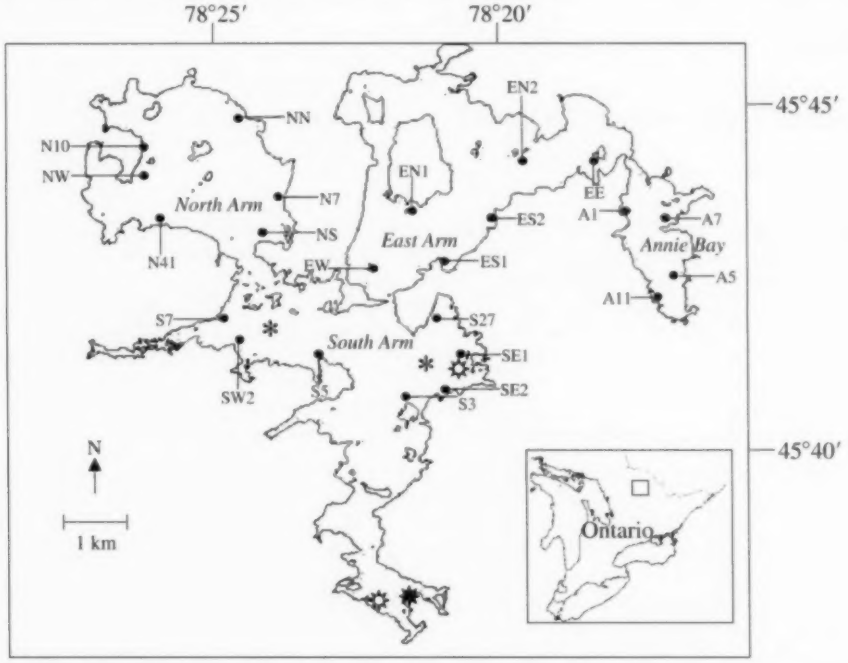
Fig. 1. Map of Lake Opeongo, Ontario, Canada. Each code marks the position of a littoral station at which water temperature was recorded. The offshore strings of thermistors in South Arm are marked by "*." The weather station for 1970–1974 is marked with a closed star and the two weather stations for 1998–1999 are marked with open stars.



main basin (Horsch and Stefan 1988; Monismith et al. 1990; James and Barko 1999). Seasonal changes in inshore–offshore temperature gradients, however, have received little attention.

Exchanges between inshore and offshore waters, however, are mostly driven by winds, either directly or indirectly. Spigel and Imberger (1980) proposed a model of wind-driven thermocline movements that is based on the relative stability of water-column stratification compared with the tangential force of the wind on the lake surface. This model distinguishes four regimes: (1) very weak thermocline (relative to wind mixing), where water mixes easily throughout the basin and stays essentially homogeneous; (2) weak thermocline, where large thermocline displacement and tilting occur and may result in hypolimnetic upwelling at the upwind end of the basin; complete vertical mixing may result in a longitudinally stratified basin with warmer water downwind; (3) sharp thermocline subject to internal seiching under strong winds; (4) very sharp thermocline, where buoyancy dominates all processes and internal waves have short periods and small amplitudes. In temperate lakes, regime 1 would occur briefly at spring and fall turnover and result in low spatial and temporal variability in water temperature, both inshore and offshore. Regime 2 would occur during spring warming and fall cooling and result in maximum spatial and temporal variability in water temperature in the littoral zone. Regime 3 would occur during summer and result in low variability in the shallow littoral zone but potentially higher temporal and perhaps spatial variability in the mid to deep littoral zone, depending on the depth of the thermocline and the amplitude of internal waves. Regime 4 has not been observed in lakes (Spigel and Imberger 1980), but a deep and very stable thermocline would presumably lead to minimal spatial and temporal variability in water temperature throughout the littoral zone. Spigel and Imberger's model suggests that the magnitude of spatial and temporal variability in water temperature in the littoral zone of lakes should vary seasonally. It also suggests that, during regime 2 (spring and fall), water temperatures should be cooler and more variable at upwind than at downwind sites.

The objective of this study was to conduct an extensive examination of some of the predictions of this model. Such empirical tests are rare in lakes other than the Great Lakes. Specifically, we use data from the four morphometrically distinct basins of Lake Opeongo to test whether (*i*) mean surface temperature and the temporal variability in surface temperature differ between inshore and offshore sites, (*ii*) the magnitude of spatial and temporal variability in inshore water temperature varies seasonally, and (*iii*) mean water temperature and the temporal variability in water temperature vary systematically from the upwind to the downwind end of a basin.

Materials and methods

Study site

Lake Opeongo (Fig. 1) is a dimictic lake located in Algonquin Park, Ontario, on the Canadian Shield (45°42′N, 78°22′W). The total surface area of the lake is 58.2 km^2, with a mean depth of 14.8 m. Lake Opeongo has four separate basins of different shapes, sizes, and depths (Table 1). South, East, and North arms are the largest and deepest basins (area 14–22 km^2, maximum depth 44–50 m). South and East arms are elongated basins (length/breadth >5),

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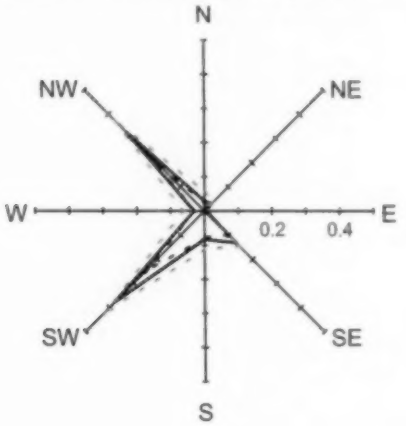
Table 1. Morphometric characteristics of Lake Opeongo basins.

Basin	Surface area (km^2)	Mean depth (m)	Maximum depth (m)	Longest axis (km)	Length:breadth ratio
Annie Bay	4.4	9.6	24	4.2	6
North Arm	14.0	15.2	44	4.8	1.4
South Arm	22.1	14.6	50	5.8	5
East Arm	18.1	16.3	44	6.9	6

Fig. 2. Proportion of total wind speed blowing from each cardinal direction from May to October 1970–1974. The average monthly proportions of wind speed from each direction are joined by a solid line; minima and maxima are joined by dashed lines.



while North Arm is more circular (length/breadth 1.4). Annie Bay is a much smaller elongated basin (area 4.4 km^2, mean depth <10 m, length/breadth 6). The shallow littoral zone ranges from rocky to sandy bottom, depending on the degree of wind exposure at each site. There were no macrophytes growing at any of the sites sampled.

Wind data

From 1970 to 1974, wind speeds and directions were collected hourly from May to October at the field station located at the southern end of the lake (closed star in Fig. 1). Wind data in 1998–1999 were collected intermittently at two positions on the lake. One weather station (model GroWeather Standard, Davis Instruments Corp., Hayward, Calif.) was mounted ~4 m above the water surface, on a shoal (<2 m depth) between the Cameron Islands in the eastern portion of South Arm (open star in Fig. 1). The station was located 25 m from the shore of both islands. A second weather station (model WS-2000, Rainwise Inc., Bar Harbour, Maine) was installed in the same way, on a shoal about 50 m east of an island at the southern end of the lake, less than 1 km from the field station (open star in Fig. 1). Technical problems and vandalism resulted in incomplete wind data sets for both 1998 and 1999.

Predominant wind directions were calculated from historical and recent measurements by weighting the winds blowing from eight directions (N, NE, E, SE, S, SW, W, NW) by their respective wind speeds (in $km \cdot h^{-1}$).

Site exposure to winds

Site exposure was measured in two ways. The effective fetch of a site (F_{eff}, in metres) was calculated according to Håkanson and Jansson (1983), as the weighted average distance over open water along the predominant wind direction. On Lake Opeongo, the predominant winds blow from two directions (NW and SW; Fig. 2). Using the detailed wind data available from May to October 1970–1974 (Fig. 2), we weighted the fetches measured along eight directions (N, NE, E, SE, S, SW, W, NW; F_d, in metres) by the proportion of wind speed blowing from each direction (w_d is the total wind speed blowing from a given direction d, from May to October):

$$F_{eff} = \sum_d (F_d \times w_d) / \sum_d w_d$$

In the elongated basins (South Arm, East Arm, Annie Bay), where winds are likely to be channeled by surrounding topography, fetch was also calculated along the main axis of the basin (F_{axis}, in metres). All fetches were measured using enhanced 1:10 000 maps produced by the Ontario Ministry of Natural Resources for each basin of Lake Opeongo.

Water temperature

Stowaway temperature loggers (models STEB08 and STEB16, response time <15 min; model TBI32-05+37, response time <3 min; Hoskin Scientific, Burlington, Ont.) recorded water temperature at 16-min intervals from May to October 1998 and from May to June 1999 at 23 sites throughout the littoral zone of the four basins of Lake Opeongo (Fig. 1). All sites were located on the edge of the main basins (i.e., none of the sites were located in small embayments). The temperature loggers were anchored within 15 m of shore on cement blocks ~40 cm above the sediment surface, at depths ranging from 1.3 to 2.4 m (Table 2). There were three exceptions in South Arm in 1998 (S7, S5, S3)—the shallow loggers failed or were lost, and temperature data measured at 3.1–3.9 m were used (Table 2). Our data suggest that the phenomena recorded by these deeper temperature loggers were similar to those recorded by shallower recorders. The mean water temperature and short-term variability were similar at two downwind sites in 1998 (S27 at 1.6 m depth, S3 at 3.4 m depth) and were clearly different from values at upwind sites (Table 2). Moreover, the relationships found in South Arm in 1998 between mean water temperature, short-term temperature variability, and fetch were corroborated in several other basins in 1998 and 1999. Thus, inclusion of data fom these three deeper stations in our analysis does not bias our conclusions.

Upon retrieval, the accuracy and precision of all instruments were determined as follows: (*i*) all instruments were set to record temperatures at 2-min intervals; (*ii*) all instruments were maintained at room temperature (23°C) for 24 h, moved to a refrigerator and maintained at 4°C for 24 h, and then re-exposed to room temperature for a further 24 h; (*iii*) exposure temperatures were recorded hourly with a high-precision mercury thermometer and (*iv*) a correction factor to insure the accuracy of each instrument was estimated (= mean difference between exposure temperature and instrument temperature) and applied to all readings from that instrument. The resolution of these instruments is about 0.15°C in the range of temperatures that were measured (Hoskin Scientific), but discrepancies between the corrected readings and the reading of the mercury thermometer were <0.1°C. The precision of each instrument was estimated from the variation in its readings when exposed to constant temperatures—this was typically <0.05°C.

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Fig. 3. Water temperature at littoral station S7 on the western end of South Arm, Lake Opeongo, during 1998. (*a*) Water temperature measurements were collected at 16-min intervals and are shown as open circles. The 2-day moving average (RMS_{2days}) is shown as a solid line. (*b*) Residuals of water temperature from the 2-day moving average. These residuals were used to calculate short-term (<2 days) temporal variability.

Table 2. Depth of measurement, degree of exposure (F_{eff}, effective fetch; F_{axis}, fetch along the main axis of the basin), and thermal conditions (mean water temperature (°C) and short-term temperature variability) in spring, summer, and fall at the littoral sites studied.

						Spring		Summer		Fall	
Year	Basin	Site	Depth (m)	F_{eff} (m)	F_{axis} (m)	Mean temperature	Temporal variability	Mean temperature	Temporal variability	Mean temperature	Temporal variability
1998	Annie Bay	$A1^U$	1.3	412	106	16.4	0.26	21.4	0.15	14.4	0.15
		$A11^U$	1.4	184	463	16.8	0.72	21.9	0.17	14.9	0.11
		$A7^D$	1.6	538	894	15.9	0.87	21.5	0.34	14.5	0.28
		$A5^D$	1.7	959	848	16.7	0.47	21.8	0.21	14.8	0.18
		Mean (±SD)				**16.5 (0.4)**	**0.6 (0.3)**	**21.7 (0.3)**	**0.22 (0.08)**	**14.6 (0.2)**	**0.18 (0.07)**
	North Arm	$N10^U$	1.5	458	0	15.7	0.54	21.4	0.10	14.8	0.05
		$N41^U$	1.7	686	634	15.8	0.53	21.4	0.12	14.7	0.07
		$N7^D$	1.4	1481	2848	16.8	0.30	21.8	0.16	14.7	0.08
		Mean (±SD)				**16.1 (0.6)**	**0.5 (0.1)**	**21.5 (0.3)**	**0.13 (0.03)**	**14.7 (0.04)**	**0.07 (0.02)**
	South Arm	$S7^U$	3.1	422	0	12.9	2.28	20.3	0.20	14.5	0.11
		S5	3.9	793	1991	13.8	1.63	21.3	0.15	14.8	0.03
		$S3^D$	3.4	1045	2675	15.3	0.40	21.0	0.10	14.7	0.05
		$S27^D$	1.6	1124	160	15.8	0.54	21.5	0.54	14.8	0.19
		Mean (±SD)				**14.4 (1.3)**	**1.2 (0.9)**	**21.0 (0.5)**	**0.2 (0.2)**	**14.7 (0.2)**	**0.09 (0.07)**
1999	North Arm	NW^U	1.9	738	0	13.9	0.22				
		NS^D	2.0	1330	1722	14.0	0.17				
		NN^D	2.4	1705	2140	15.3	0.32				
		Mean (±SD)				**14.4 (0.8)**	**0.24 (0.08)**				
	South Arm	$SW2^U$	2.0	701	0	12.2	0.71				
		$S7^U$	2.4	556	955	12.9	0.29				
		$SE2^D$	2.1	1587	2140	13.0	0.23				
		$SE1^D$	1.9	1655	2008	13.3	0.25				
		Mean (±SD)				**12.8 (0.4)**	**0.4 (0.2)**				
	East Arm	EW^U	2.0	341	0	12.3	1.72				
		ES1	2.1	1081	796	12.8	0.80				
		EN1	2.0	1070	1802	13.1	0.57				
		ES2	2.1	1607	2028	13.3	0.29				
		$EN2^D$	2.0	1015	2001	13.6	0.15				
		EE^D	2.0	1233	1842	13.6	0.24				
		Mean (±SD)				**13.1 (0.5)**	**0.6 (0.6)**				

Note: The depths at which temperature was measured are listed for the beginning of the year; water levels dropped by about 0.25 m from May to October in Lake Opeongo. Short-term temperature variability is calculated as the residual mean square of a 2-day moving average (RMS_{2days}). Values in bold are basin-wide mean temperature and mean temporal variability. Site locations are shown in Fig. 1; upwind and downwind sites are identified by the superscripts U and D, respectively.

Data analysis

The water temperature data series were divided into spring, summer, and fall periods for the analyses. Spring and fall were defined as those dates on which the average difference between the surface (4 m) and bottom (15 m) temperatures, calculated with a 2-day moving average, were less than 10°C. Summer was defined as those dates on which the average difference was greater than 10°C. This somewhat arbitrary cut-off point generally agrees with the calendar definition of seasons in 1998, and allows periods to be defined with similar strengths of thermal stratifications in different years. Using this criterion, the spring, summer, and fall periods in 1998 were defined as 9 May – 21 June, 22 June – 8 September, and 9 September – 27 October, respectively. Spring warming occurred much more rapidly in 1999, shortening the spring period to 8 May – 31 May, almost one-half as long as in 1998. Offshore temperature profiles were recorded using two strings of 8–10 Stowaway temperature recorders (models STEB08 and STEB16 in 1998; model TBI32-05+37 in 1999) anchored in South Arm (positions indicated by asterisks in Fig. 1).

Mean water temperature and short-term temporal variability were calculated for each period (spring, summer, fall) at each site. Short-term temporal variability was calculated as the residual mean square from a 2-day moving average (RMS_{2days}; Fig. 3). The 2-day moving average removes the seasonal changes in water temperature (e.g., spring warming, fall cooling) and focuses the analysis on short-term temporal variability due to diurnal cycles and rapid changes in atmospheric conditions (~0.5–48 h). Other temporal scales of variability, which could also be important to the organisms inhabiting the littoral zone, will be described elsewhere.

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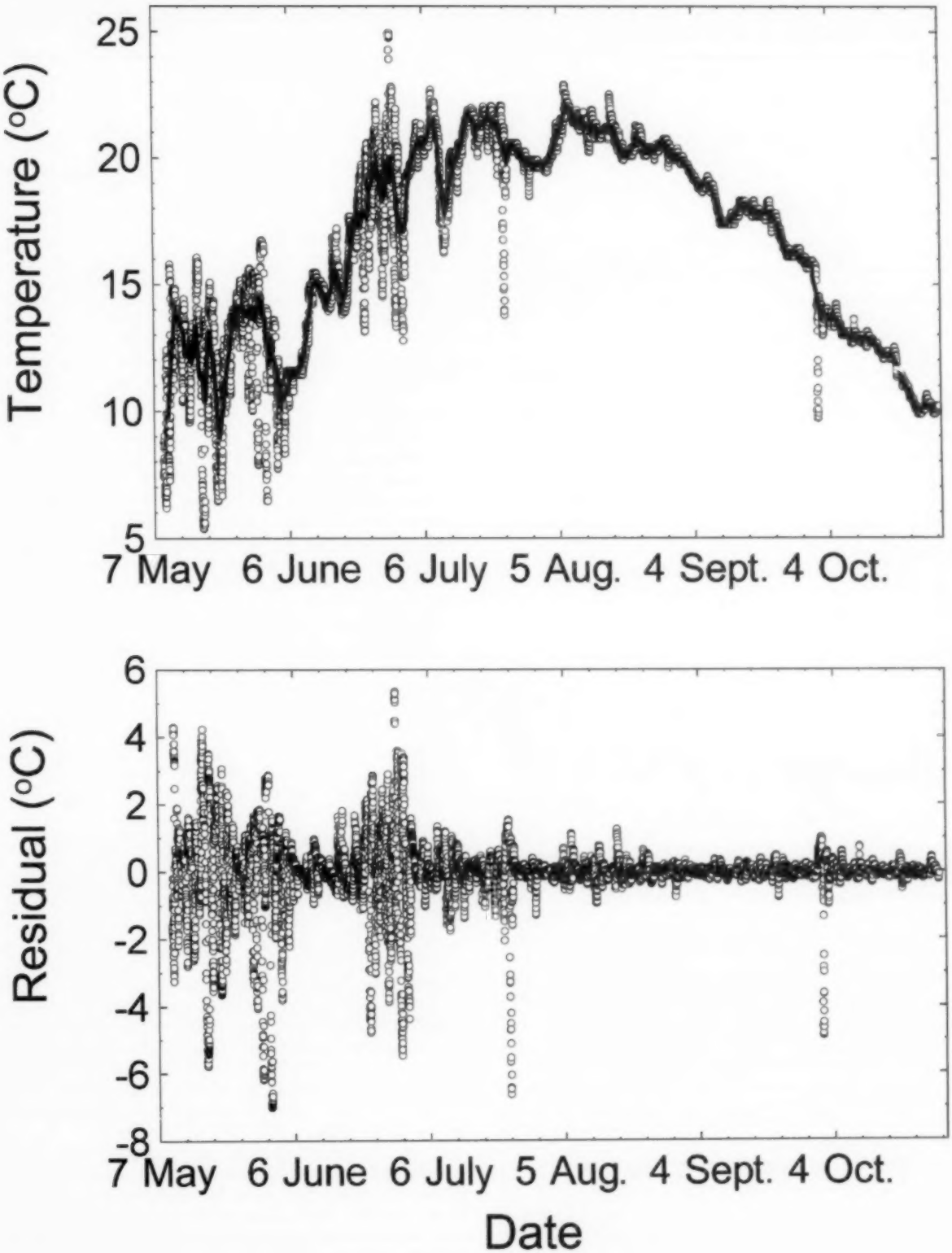

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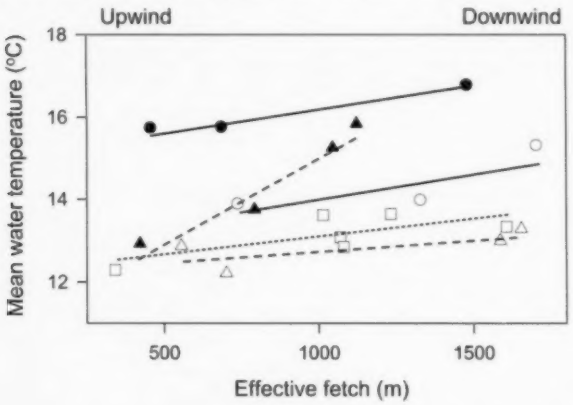
Table 3. Relationships between mean water temperature and site exposure and between short-term temporal variability (RMS_{2days}) and site exposure in the four basins of Lake Opeongo.

		Mean water temperature						Short-term temporal variability					
		Spring		Summer		Fall		Spring		Summer		Fall	
Basin	Year	F_{eff}	F_{axis}	F_{eff}	F_{axis}	F_{eff}	F_{axis}	F_{eff}	F_{axis}	F_{eff}	F_{axis}	F_{eff}	F_{axis}
Annie Bay	1998	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
North Arm	1998	+ (0.12)	—	+ (0.17)	—	ns	—	– (0.12)	—	+ (0.02)	—	+ (0.28)	—
	1999	+ (0.38)	—	—	—	—	—	ns	—	—	—	—	—
South Arm	1998	+ (0.03)	ns	+ (0.17)	ns	+ (0.20)	ns	– (0.03)	ns	ns	ns	ns	– (0.16)
	1999	ns	+ (0.08)	—	—	—	—	ns	– (0.10)	—	—	—	—
East Arm	1999	+ (0.10)	+ (0.01)	—	—	—	—	– (0.04)	– (0.003)	—	—	—	—

Note: Site exposure is measured as effective fetch (F_{eff}) or as fetch along the main axis of the basin (F_{axis}). The signs of all statistically significant relationships are shown (+ or –) with their P values in parentheses. Significance levels used are $\alpha = 0.4$ in North Arm ($n = 3$), $\alpha = 0.2$ in South Arm and Annie Bay ($n = 4$), and $\alpha = 0.05$ in East Arm ($n = 6$) (see Materials and methods for details); ns indicates a non-significant relationship; "—" indicates that the relationship could not be tested.

Fig. 4. Relationships between spring mean water temperature and effective fetch at littoral sites in different basins of Lake Opeongo. Circles and solid lines indicate North Arm ($n = 3$), triangles and broken lines indicate South Arm ($n = 4$), and squares and dotted line indicate East Arm ($n = 6$). Filled symbols indicate 1998 (9 May – 21 June); open symbols indicate 1999 (8 May – 31 May). The P values for each regression line are shown in Table 3.



Regression analysis (Zar 1984) was used to test whether mean water temperature and short-term temporal variability in water temperature in each basin were related to site exposure. In basins for which data were only available from three or four sites, unreasonably high fits (i.e., correlation coefficients of 0.997 and 0.95 for $n = 3$ and 4, respectively; Table B.16 in Zar 1984) would be required before a relationship would be declared statistically significant using the traditional $\alpha = 0.05$. This would result in low power, or a high probability of accepting a false null hypothesis (i.e., high Type II error). To reduce Type II error and equalize among lake basins the strengths of relationships that are considered statistically significant (equivalent to $r = 0.80$), we chose (a priori) significance levels of $\alpha = 0.05$ in East Arm ($n = 6$ sites), $\alpha = 0.2$ in South Arm and Annie Bay ($n = 4$ sites), and $\alpha = 0.4$ in North Arm ($n = 3$ sites). Both the effective fetch (F_{eff}) and the fetch along the main axis of the basin (F_{axis}) were used as measures of site exposure in South Arm, East Arm, and Annie Bay; only F_{eff} was used in the more circular North Arm (Table 1). The regression analyses were supplemented by one-tailed t tests that compared mean water temperature at upwind and downwind sites in each basin. All statistical analyses were performed using Statistica for Windows, version 5.

Results

Prevailing winds and site exposure

The prevailing winds on Lake Opeongo from May to October 1970–1974 blew from the southwest (on average, 35% of total wind speed, with a range of 27–46% in different months and years) and northwest (on average, 31% of total wind speed, with a range of 27–41%; Fig. 2). The predominant wind directions were generally similar in 1998 and 1999, with similar proportions of wind speed from the south, southwest, and west in 1998 (period of measurement 28 July – 25 August) and similar proportions of wind speed from the southwest and west in 1999 (20–30 June, 16–21 July, and 13 September – 4 October).

The sampling sites in each basin were chosen to vary in their degree of exposure to the predominant winds. In each basin, the effective fetch (F_{eff}) varied 4- to 5-fold among sampling sites, and the range of values for fetch along the main axis (F_{axis}) was even greater (Table 2). These two measures of site exposure were significantly correlated in East Arm ($r = 0.81$, $P = 0.048$) but not in South Arm ($P = 0.13$) or Annie Bay ($P = 0.41$).

Comparison of water temperature at inshore and offshore sites

Offshore water temperature was measured during spring and summer using two strings of temperature loggers positioned at either end of South Arm. In spring 1998, the mean surface temperature (shallowest temperature logger) was 14.0°C at the western end of South Arm (3.8 m depth) and 14.2°C at the eastern end of South Arm (5.8 m depth). These offshore temperatures were intermediate compared with temperatures measured at littoral sites at the western (upwind; sites S7 and S5, 12.9–13.8°C) and eastern (downwind; sites S3 and S27, 15.3–15.8°C) ends of the basin (Table 2). Water temperatures were more homogeneous in the summer, with a maximum inshore–offshore difference of ~1°C (inshore 20.3–21.5°C; offshore 20.9–21.1°C).

In 1998, temporal variability was also intermediate at offshore stations; spring values of RMS_{2days} were 1.17 at the western string and 0.65 at the eastern string, compared with values of 0.40–2.28 at littoral stations (Table 2). In the summer, surface water temperature was less variable, both inshore and offshore (RMS_{2days}: inshore = 0.1–0.5; offshore at

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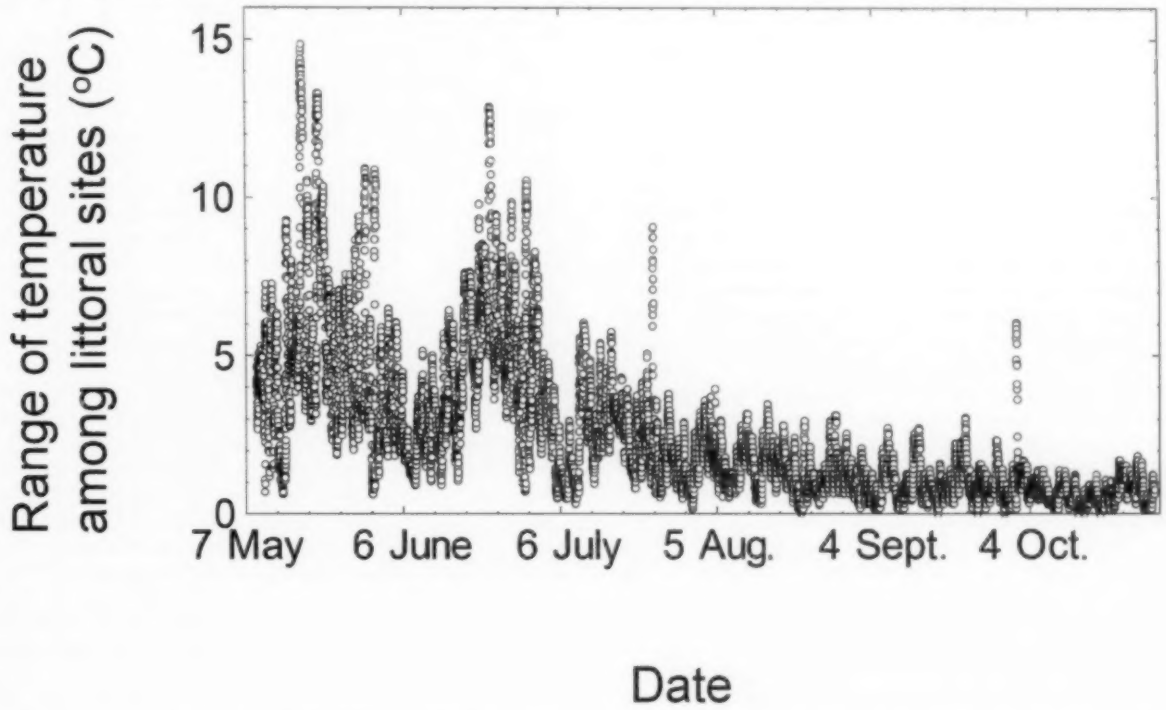
Table 4. Range in water temperature measured among littoral sites within each basin of Lake Opeongo.

			Range in water temperature within basin (°C)					
			Spring		Summer		Fall	
Year	Basin	*n*	Median	Maximum	Median	Maximum	Median	Maximum
1998	Annie Bay	4	1.7	8.2	0.9	4.4	0.8	2.3
	North Arm	3	1.8	11.0	0.8	6.5	0.3	1.6
	South Arm	4	4.0	14.8	1.8	12.9	0.8	7.8
1999	North Arm	3	1.9	6.7				
	South Arm	4	1.7	7.6				
	East Arm	6	2.0	9.5				

Note: The median and maximum instantaneous ranges in water temperature (measured at intervals of 16 min) are shown for each basin for the spring, summer, and fall periods; "*n*" is the number of littoral sites being compared in each basin. See Fig. 5 for the full series of instantaneous measurements in South Arm in 1998.

Fig. 5. Maximum differences in water temperature observed between four littoral sites in South Arm (S7, S5, S3, S27; Fig. 1) during 1998. Each point represents instantaneous temperature differences measured at 16-min intervals from 9 May to 27 October.



the western end = 0.31). Offshore water temperatures were more variable at the eastern end of South Arm (RMS_{2days} = 1.15), because the shallowest temperature recorder was anchored at 6 m, within the zone influenced by internal waves (data not shown). Measurements of offshore thermal conditions do not reflect the extremes experienced in different portions of the littoral zone.

Spatial variability in water temperature

Mean littoral temperature in the spring differed by 1.5–2°C among the four basins of Lake Opeongo (Table 2). On average, the littoral zone was 1.5°C cooler in South Arm than in North Arm (1998 and 1999) and 2°C cooler in South Arm than in Annie Bay (1998). The littoral zones of the two large elongated basins (South and East arms) had similar mean water temperatures (12.8 and 13.1°C, respectively, in 1999). Temperature differences among basins decreased as the summer progressed (maximum difference in summer mean water temperatures among basins of 0.7°C) and disappeared in the fall (difference <0.1°C; Table 2).

Within basins, mean water temperature in the spring varied by 1–2.9°C among littoral sites (Table 2). Spring mean water temperature was positively related to site exposure (F_{eff} and (or) F_{axis}) in all basins except Annie Bay ($P > 0.6$; Table 3; Fig. 4), suggesting that littoral sites were warmer downwind than upwind. This result was supported by combining one-tailed *t* tests that compared mean water temperature at upwind and downwind sites (*t* tests: $P = 0.07$ in Annie Bay; $P = 0.03$

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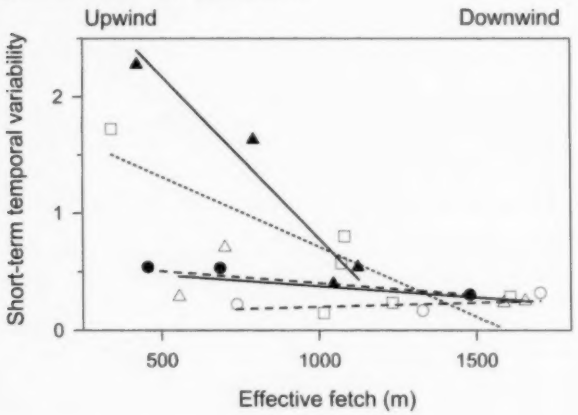
Fig. 6. Relationships between spring short-term (<2 days) temporal variability (RMS_{2days}) and effective fetch at littoral sites in different basins of Lake Opeongo. Circles and broken lines indicate North Arm (n = 3), triangles and solid lines indicate South Arm (n = 4), and squares and dotted line indicate East Arm (n = 6). Filled symbols indicate 1998 (9 May – 21 June); open symbols indicate 1999 (8 May – 31 May). The P values for each regression line are shown in Table 3.



in South Arm in 1998; P = 0.13 in South Arm in 1999; P = 0.02 in East Arm; combined probability, $P < 0.01$; Sokal and Rohlf 1981, p. 779). The relationships between mean water temperature and site exposure were also statistically significant in South and North arms in the summer and in South Arm in the fall (Table 3), but water temperatures became more uniform, with differences of ≤0.5°C among littoral sites in the summer and fall (Table 2). Downwind sites were generally warmer than upwind sites in large basins, especially in the spring (Table 2; Fig. 4).

Within-basin spatial variability in littoral temperature was even more dramatic when individual measurements (every 16 min) rather than seasonal averages were considered (Table 4; Fig. 5). Littoral sites within a basin differed in water temperature in the spring by as much as 6.7–14.8°C (Table 4), with median differences of 1.7–4.0°C. A dramatic difference in water temperature was directly observed on the day the temperature recorders were set up in North Arm in 1998. Using a YSI temperature meter, water temperatures ranging from 8.5°C (close to station NN) to 16.0°C (station N10) were measured while travelling around the basin (<1 h). As the season progressed, water temperature became more uniform, but differences of more than 2°C were still observed sporadically (Table 4). Large differences in water temperature among littoral sites were not uncommon, especially in the spring, and could persist for several hours at a time (Fig. 5).

Short-term temporal variability (<2 days) in water temperature

Water temperature was more variable temporally in large elongated basins (South and East arms) than in the more circular North Arm and the smaller Annie Bay (Table 2). In spring 1998, the average short-term variability in water temperature, measured as the residual mean square from a 2-day moving average (RMS_{2days}; see Materials and methods), reached a value of 1.21 in South Arm, compared with 0.58 in Annie Bay and 0.46 in North Arm. In the spring of 1999, East Arm littoral sites were, on average, the most variable (0.63), followed by South Arm (0.37) and North Arm (0.24). Short-term temporal variability in water temperature decreased through the summer and fall (Fig. 3; Table 2).

Table 5. Relationships between short-term variability in water temperature (RMS_{2days}) and mean water temperature at littoral sites in the four basins of Lake Opeongo.

Basin	Year	Spring	Summer	Fall
Annie Bay	1998	ns	ns	ns
North Arm	1998	– (0.002)	+ (0.18)	– (0.37)
	1999	+ (0.26)	—	—
South Arm	1998	– (0.03)	ns	ns
	1999	– (0.06)	—	—
East Arm	1999	– (0.001)	—	—

Note: The signs of significant relationships are indicated (+ or –), with their P values in parentheses. Significance levels used are α = 0.4 in North Arm (n = 3), α = 0.2 in South Arm and Annie Bay (n = 4), and α = 0.05 in East Arm (n = 6) (see Materials and methods for details); ns indicates a non-significant relationship; "—" indicates that the relationship could not be tested.

Within basins, temperature variability at littoral sites ranged as little as 1.5- to 2-fold in North Arm (1998 and 1999) but as much as 5.8-fold in South Arm (1998) and 11.6-fold in East Arm (1999; Table 2). Short-term temperature variability clearly declined as the season progressed (e.g., from 1.21 in the spring to 0.09 in the fall in South Arm in 1998), but the relative difference in temperature variability among littoral sites did not change (e.g., temperature variability at different sites in South Arm ranged 5.8-, 5.6-, and 5.3-fold, respectively, in the spring, summer, and fall of 1998). However, sites where water temperature was highly variable in the spring were not necessarily the ones with the highest variability later in the season (Table 2).

Short-term temperature variability in the spring was negatively related to site exposure in all basins except Annie Bay ($P > 0.2$; Table 3; Fig. 6). As the season progressed, this relationship dampened and, in North Arm, even reversed (Table 3). In general, the warmer downwind sites in large lake basins also had less variable water temperatures (Table 5).

Discussion

Inshore–offshore differences in water temperature

Large bodies of water are expected to warm up more slowly than small basins in the spring, owing to their larger volume. Differential heating of the shallower littoral zone, however, should result in very different rates of heating of the water inshore and offshore. In Lake Opeongo, mean water temperatures in the littoral zone were found to be lower in large basins than in smaller basins (compare South and East arms with Annie Bay). Maximum temperatures in the littoral zone of large basins were also lower than the coolest littoral sites in small basins (compare site S27 in South Arm with sites in Annie Bay). This suggests that inshore–offshore mixing slows the rate of heating of water in the littoral zone of large basins. This may be the case at downwind sites but,

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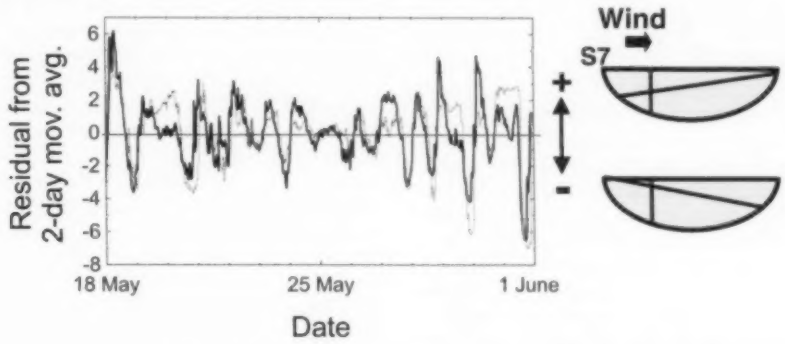
Fig. 7. Comparison of short-term variability (<2 days) between the temperature (°C) at an upwind littoral site (S7; dotted line) and the depth (m) of the 8°C isotherm measured offshore at the upwind (western) end of South Arm (solid line) during May 1998. To focus the comparison on short-term variability, both variables are presented as residuals from a 2-day moving average (mov. avg.). As the offshore 8°C isotherm (solid line in the graph) moved to shallower depths (negative residuals), the littoral temperatures (dotted line) became cooler (negative residuals). As the offshore 8°C isotherm moved deeper (positive residuals), the littoral temperature became warmer (positive residuals). The diagrams on the right show the presumed tilting of the isotherm relative to the upwind littoral site (S7) and the offshore sampling station (vertical line).



since offshore surface temperatures in South Arm were intermediate between the warmest and coolest littoral sites, offshore–inshore mixing cannot account for the very low temperatures measured at upwind littoral sites. Some preliminary data from South Arm suggest that periodic upwellings of cold bottom water contribute to slower warming of the littoral zone in the spring (see below).

Seasonal changes in temperature variability

Water temperature in the shallow littoral zone of Lake Opeongo was most variable, both spatially and temporally, in the spring and became more homogeneous as the season progressed. These results are consistent with the effects of deepening and strengthening of the thermocline predicted by Spigel and Imberger (1980). As the season progresses, more wind energy is required to disrupt the thermal structure of a lake. Such large storms do occur (e.g., 23 July and 1 October 1998) but are rare.

Not surprisingly, mean water temperature and the magnitude of short-term variability in water temperature vary greatly between years (compare 1998 and 1999 data for North and South arms). Local meteorological conditions (e.g., air temperature, wind speed, wind direction) and the timing of storm events relative to the stability of the water column determine the rate at which a thermocline develops and, ultimately, the thermal structure of a lake (e.g., Spigel and Imberger 1980; Imberger 1994). For example, in 1998, a violent storm in early June mixed the surface layer of Lake Opeongo and led to a sudden drop in surface water temperature of 4.5°C. This drop was observed in all basins and at all littoral sites, and cooled the littoral zone for several days. In 1999, a warm spring led to rapid warming of the surface water. A 10°C difference between the surface and bottom waters (our criterion for distinguishing spring from summer) developed in half the time as in the previous year. Midsummer thermocline depths in medium and large lakes (>3 km^2) have been shown to vary by as much as 5–6 m between years, depending on surface area, local wind conditions, and water clarity (Fee et al. 1996; King et al. 1999). Although accurate forecasting of meteorological conditions, and of mean water temperatures, from one year to the next is still beyond our reach, our results suggest that we can predict, at least qualitatively, which portion of the littoral zone will be warmer and will have more constant temperatures.

Gradient between upwind and downwind sites

Downwind littoral sites are generally warmer and have more constant temperatures than upwind sites. The magnitude of this difference varies among basins and diminishes as the season progresses, but the pattern is consistent.

Some preliminary data in South Arm suggest that the colder and more variable water temperatures observed at the upwind sites are due to periodic incursions of cold bottom water. In the spring of 1998, simultaneous oscillations of the 8°C isotherm depth at the western offshore site in South Arm and the water temperatures at an upwind littoral site were observed (S7; Fig. 7). As the offshore 8°C isotherm moved to shallower depths (negative residuals, solid line in Fig. 7), the littoral temperatures became cooler (negative residuals, dotted line in Fig. 7). As the offshore 8°C isotherm moved deeper (positive residuals), the littoral temperature became warmer (positive residuals). This synchrony between offshore isotherm depth and littoral temperature suggests that direct wind forcing is very important in determining littoral temperatures at upwind sites. Unfortunately, we cannot test this hypothesis further, because our weather stations failed during this sampling period and the closest weather station (Petawawa, Ont.) is located too far from the site (~90 km) to provide representative wind data. We are presently testing the link between offshore isotherm movements and littoral temperatures more rigorously, using data from South and North arms collected in 1999.

Upwellings at upwind sites are predicted by the model of Spigel and Imberger (1980) and, while well known in the North American Great Lakes (Boyce et al. 1989), have only been documented in a few intermediate-size lakes (e.g., Mortimer 1952). Such upwellings would cool the upwind portion of the littoral zone, but could also increase nutrient

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inputs and, possibly, the frequency and strength of disturbances at these sites. The frequency and ecological importance of upwellings in intermediate- and small-size lakes are still largely unknown.

Mean water temperature and short-term variability in water temperature at littoral sites vary with basin morphometry. In the spring, littoral sites were colder, on average, in the large elongated basins (South and East arms) than in either the small basin (Annie Bay) or the large circular basin (North Arm). Surprisingly, mean water temperature and short-term variability in water temperature were very similar in Annie Bay and North Arm, despite a large difference in volume. North Arm has a volume of water that is five times larger than Annie Bay and more similar to South and East arms (66 and 72% of the volume of South and East arms, respectively). North Arm is also the only basin where positive relationships between mean water temperature and short-term variability in water temperature were observed (summer 1998 and spring 1999). Based on these findings, we suggest that basin morphometry is at least as important as surface area in determining water movements and the spatial and temporal variability of water temperature at littoral sites.

Ecological impacts of temperature variability

Mean water temperature varies by as much as 3–4°C among littoral sites in Lake Opeongo and can differ by as much as 7–15°C over short periods of time. Since aquatic organisms are mostly ectotherms, their activity and physiological rates should be affected by such temperature differences. Rejwan et al. (1999) showed that the primary habitat characteristic distinguishing traditional smallmouth bass spawning areas in Lake Opeongo was littoral water temperature (high-use spawning areas were warmer than low-use or no-use areas). We also used published relationships between physiological rates and water temperature (algal growth rate from Regier et al. (1990); smallmouth bass hatching time and growth rate from Shuter et al. (1980)), to estimate the potential ecological impact of the temperature regimes observed at the coldest (upwind) and warmest (downwind) sites in South Arm (sites S7 and S27, respectively). According to these calculations, the growth rate of algae during spring 1998 (9 May – 21 June) would be ~40% higher downwind, the hatching time of smallmouth bass eggs (based on a typical egg-development period in Lake Opeongo, 25 May – 15 June) would be ~35% shorter downwind (7 instead of 11 days), and the growth of juvenile smallmouth bass (over their typical growing season in Lake Opeongo, 15 June – 15 September) would be ~30% greater downwind. These effects are largely due to a mean temperature difference in the spring of almost 3°C among sites. Warmer temperatures at downwind sites lead to faster growth of the organisms, and may allow growth to start earlier in the spring. More early growth should affect later breeding behaviour and success, but organisms at downwind sites are also more vulnerable to destructive wave disturbances, especially in the spring before the lake thermal structure stabilizes (e.g., a violent storm in early June 1998 wiped out all bass nests at the eastern end of South Arm; M.S. Ridgway, Harkness Laboratory of Fisheries Research, Aquatic Ecosystem Science Section, Ontario Ministry of Natural Resources, Peterborough, ON K9J 8M5, Canada, personal communication).

Natural temperature fluctuations, which we measured as RMS_{2days}, may also be important, but we are unaware of any model that could help us estimate their impact. Much work has been done on the effect of diurnal changes in water temperature on aquatic organisms (e.g., fish diurnal migration; Biette and Geen 1980; Diana 1983), but the physiological and ecological implications of more irregular short-term temperature variations are rarely studied. Spigarelli et al. (1982) showed that adult brown trout exposed to natural fluctuations in water temperatures from inshore Lake Michigan in May had higher lipid and muscle content, three times higher daily consumption, and twice the daily growth predicted for fish exposed to similar but constant temperatures. Fluctuating temperatures have also been shown to regulate the taxonomic and functional composition of microbial communities (e.g., Upton et al. 1990; Ogilvie et al. 1997).

The calculated differences in productivity between upwind and downwind sites presented above are contingent on the organisms immediately equilibrating their body temperature to that of the surrounding water. However, organisms of different sizes experience environmental temperature variability differently. Very small organisms, such as bacteria and algae, equilibrate their body temperature with changing water temperatures more quickly than larger organisms, such as fish (e.g., Peters 1983; Cossins and Bowler 1987). Fish up to 100 g in body mass are expected to equilibrate their body temperature to a 10°C change in water temperature in less than 15 min, while a 1-kg fish would require about 30 min and a 10-kg fish about 75 min (Peters 1983). The body temperature of smaller organisms (e.g., bacteria, algae, invertebrates) would equilibrate even faster with their surroundings, provided they were not shielded in some way from these temperature changes (e.g., buried in sediments). Based on these calculations, many of the smaller organisms living in the littoral zone would experience the temperature fluctuations we measured at 16-min intervals, whereas larger fish would be more affected by temperature shifts that persist over several hours. The anomalous temperature events we observed generally lasted several hours and would be expected to affect even the larger fish.

The spatial and temporal variability of water temperature in the littoral zone of lakes are most likely to affect fish and invertebrate species that use discrete habitats at critical periods in their life cycle (e.g., smallmouth bass). Other fish species and large benthic invertebrates can avoid unfavorable temperatures and might not be affected as directly. All littoral fish and invertebrates, however, are ultimately dependent on smaller less-mobile organisms for food, and conditions affecting the growth and productivity of bacteria, algae, and small benthic invertebrates should eventually affect higher trophic levels. The distributions of mean water temperature and temperature variability are likely to affect the productivity of littoral organisms, either directly or indirectly.

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Recovery of ovary size, follicle cell apoptosis, and HSP70 expression in fish exposed to bleached pulp mill effluent

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Abstract: We hypothesized that impaired ovarian function (e.g., reduced ovary size) in wild fish chronically exposed to bleached pulp mill effluents is related to an increased rate of apoptosis in ovarian follicle (granulosa and (or) theca) cells. In 1996, vitellogenic white sucker (*Catostomus commersoni*) collected downstream of a bleached kraft pulp mill had reduced ovary size associated with increases in ovarian cell apoptosis and stress protein (HSP70) expression when compared with fish collected from an upstream reference site. Fish sampling was repeated in 1998 following mill maintenance changes aimed at minimizing the discharge of process chemicals. In 1998, there were no differences in ovary size, ovarian cell apoptosis, or ovarian HSP70 levels between fish collected upstream and downstream of the mill. Based on the results of this and our previous work, we conclude that chronic stimulation of ovarian cell apoptosis by certain components of mill effluents may represent an important cellular mechanism responsible for reductions in ovary size, and potentially other integrated reproductive responses, in female fish exposed to pulp mill effluents.

Résumé : Notre hypothèse veut que la détérioration du fonctionnement des ovaires (e.g. la taille réduite des gonades) chez les poissons soumis à une exposition chronique aux effluents de papetières avec blanchiment de la pâte soit reliée à un taux accru d'apoptose des cellules (de la granuleuse et (ou) de la thèque) du follicule ovarien. En 1996, des Meuniers noirs (*Catostomus commersoni*) en vitellogenèse capturés en aval d'une papetière utilisant le procédé Kraft avec blanchiment de la pâte avaient des ovaires de taille réduite, une apoptose accrue des cellules ovariennes et une expression plus marquée de la protéine du stress (HSP70) que des poissons provenant d'un site-témoin situé en amont. Un nouvel échantillonnage a été réalisé en 1998 après que des changements eurent été apportés au fonctionnement de l'usine dans le but de minimiser le déversement des produits chimiques utilisés dans le traitement. Il n'y avait alors aucune différence entre les tailles des ovaires, les taux d'apoptose des cellules ovariennes ou les concentrations d'HSP70 dans les ovaires des poissons de l'amont et de l'aval de l'usine. Sur la foi de ces résultats et de nos travaux antérieurs, il appert que la stimulation chronique de l'apoptose dans les cellules ovariennes par des constituants de l'effluent des papetières est un important mécanisme cellulaire responsable de la réduction de la taille des ovaires, et probablement aussi d'autres effets globaux sur la reproduction, chez les poissons femelles exposés aux effluents de ces usines.

[Traduit par la Rédaction]

Introduction

It is well established that chronic exposure of wild fish to bleached kraft pulp mill effluents (BKME) causes alterations in reproductive function (Sandström 1996; Munkittrick et al. 1998). Detailed studies conducted since the late 1980s in northern Ontario populations of white sucker (*Catostomus commersoni*) exposed to BKME have shown that females exhibit a characteristic suite of effects, the most consistent being reduced ovarian size, decreased fecundity with age, smaller egg size, delayed sexual maturation, alterations in plasma sex steroid levels, and induction of hepatic cytochrome P450 dependent detoxification enzyme activities (McMaster et al. 1991; Munkittrick et al. 1991; Van Der Kraak et al. 1998). The effects on ovarian physiology were consistent for different stages of oogenesis, different pulp mill locations, and different years and following changes in effluent treatment aimed at reducing environmental impacts of BKME in aquatic species (Munkittrick et al. 1994, 1998; McMaster et al. 1996). However, the underlying cellular mechanisms responsible for such disruptions in ovarian function remain unclear.

Atresia is a hormonally controlled degenerative process by which ovarian follicles are eliminated prior to ovulation in all vertebrates (Byskov 1978). Atresia can be either a normal physiological process involved in selection of competent follicles (Hsueh et al. 1994) or an abnormal process occurring in response to environmental stressors such as poor nutrition, hypoxia, or temperature extremes (Saidapur 1978). Since a variety of environmental stressors are known to in-

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duce atresia in fish (Saidapur 1978), the extent of atresia during oogenesis may represent a significant determinant of the overall reproductive success in wild fish populations (N'Da and Déniel 1993). Apoptosis of granulosa cells that provide hormonal support for the oocyte is the normal mechanism by which atresia occurs in mammals, birds (Hsueh et al. 1994), and possibly fish (Janz and Van Der Kraak 1997). Apoptosis plays a critical role in the development and homeostasis of all metazoan animals (Steller 1995) and is also believed to represent a molecular "control point" in toxicity (Corcoran et al. 1994; Tilly et al. 1997; McConkey 1998). The consistent reductions in ovarian growth and development in wild fish exposed to BKME led us to hypothesize that certain effluent components stimulate apoptosis in ovarian follicular (granulosa and (or) theca) cells and thus may represent a fundamental cellular mechanism involved in impaired ovarian function.

Members of the heat shock protein (HSP) family, also known as stress proteins, have been used as biochemical indicators of environmental stress in aquatic species (Iwama et al. 1998; Feder and Hofmann 1999). In fish, the HSP70 family has been studied most extensively and it has been determined that expression of piscine HSP70s is induced in response to various environmental stressors including toxicants (Ryan and Hightower 1994; Williams et al. 1996). BKME exposure has been reported to induce HSP70 expression in liver (Vijayan et al. 1998) and ovary (Janz et al. 1997) of fish. Increased ovarian HSP70 expression following BKME exposure may be relevant to ovarian physiology of fishes, since recent studies have shown that inducible HSP70 protects mammalian cells from stress-induced apoptosis (Mosser et al. 1997; Mallouk et al. 1999) and HSP70 is associated with inhibition of steroidogenesis and mediation of luteal regression in the mammalian ovary (Khanna et al. 1995).

The main objective of this study was to determine ovarian cell apoptosis, gonadosomatic index (GSI), and HSP70 expression during the vitellogenic (growth) stage of ovarian development in white sucker in order to compare fish collected upstream and downstream of a bleached kraft pulp mill located in Smooth Rock Falls, Ontario. Plasma testosterone and 17β-estradiol concentrations were determined to examine whether elevated ovarian follicular cell apoptosis was related to alterations in steroidogenesis. Hepatic ethoxyresorufin *O*-deethylase (EROD) activity was also determined as a specific marker of exposure to aryl hydrocarbon receptor (AhR) agonists. Fish were collected in two different years, before and after the pulp mill conducted a number of improvements aimed at eliminating the release of process chemicals. This was of particular interest due to new regulations for Canadian pulp and paper mills, implemented in 1993, which have resulted in partial recovery of reproductive responses at some mills (reviewed in Munkittrick et al. 1998).

Materials and methods

Study site

Production capacity of the bleached kraft pulp mill at Smooth Rock Falls is 170 000 air-dried metric tons of elemental chlorine free kraft pulp per year (465 $t \cdot day^{-1}$) using continuous-flow digestion. The kraft primary bleaching process was converted to 100% ClO_2 substitution in 1992, and secondary treatment of effluent was initiated in October 1994. Of importance to this study, during a regularly scheduled mill shutdown in 1997 (between fish collections conducted in 1996 and 1998), a number of unspecified changes were made to minimize the release of process chemicals. Effluent is discharged into the Mattagami River approximately 0.15 km downstream of the pulp mill by a submerged diffuser at a rate of approximately 0.8 $m^3 \cdot s^{-1}$.

Fish collection and sampling

Vitellogenic white sucker were captured using overnight gillnet sets in September 1996 and September 1998. Fish were collected approximately 4 km downstream of the mill and at an upstream reference site. A dam that prevents movement of fish between sites separates the upstream and downstream locations. Blood was collected for steroid hormone analysis from the caudal artery using a syringe. The ovaries were removed and weighed and approximately 100 follicles were frozen immediately in liquid nitrogen for determination of apoptotic DNA fragmentation and HSP70 expression. The GSI was calculated as 100 × [ovary weight/(body weight – ovary weight)].

Apoptotic DNA fragmentation

Total genomic DNA was extracted and phenol–chloroform purified from a pool of eight ovarian follicles from each fish. DNA (2 µg) from each sample was 3′-end labeled with [α^{32}P]dideoxy-ATP (Amersham; 3000 $Ci \cdot mmol^{-1}$ for 1996 and 1500 $Ci \cdot mmol^{-1}$ for 1998 (1 Ci = 37 GBq)) using terminal deoxynucleotidyl transferase (Boehringer Mannheim; 25 $U \cdot mL^{-1}$) as described previously (Janz and Van Der Kraak 1997). Radiolabeled DNA was loaded onto 2% agarose gels (1 $\mu g \cdot lane^{-1}$) and fractionated by electrophoresis. A 123 base pair DNA ladder (Gibco BRL) was also 3′-end labeled and run on each gel as a standard. Following electrophoresis, the gel was exposed to Fuji or Kodak X-Omat film for 2–8 h at –80°C. Portions of each lane corresponding to low molecular weight DNA (<15 kilobase pairs) were cut from the gel with a scalpel and the extent of apoptotic DNA fragmentation was quantified by liquid scintillation counting.

Western blotting

HSP70 was detected in ovarian follicles by Western immunoblotting as described previously (Janz et al. 1997), with some modifications for the 1998 samples. Approximately 50 follicles from individual fish were used to isolate total ovarian cell protein. Protein (40 $\mu g \cdot lane^{-1}$) was separated using SDS–PAGE with 10% acrylamide minigels (1996) or 7.5–20% acrylamide gradient gels (1998), and protein was transferred to nitrocellulose membranes. Immunoblotting of 1996 samples was performed using a polyclonal rabbit anti-rainbow trout HSP70 antibody (Forsyth et al. 1997). For 1998 samples, immunoblotting was performed using a monoclonal anti-HSP70 antibody (Sigma H5147). Immunoreactive bands were detected using enhanced chemiluminescence (Pierce). The optical density of bands was determined using NIH image software.

Steroid hormone and EROD assays

Plasma concentrations of testosterone and 17β-estradiol were determined using validated radioimmunoassays as described previously (McMaster et al. 1992). Archived plasma samples were ether extracted and analyzed in the same assays to facilitate comparisons across the two collections. Hepatic EROD activities were measured in postmitochondrial supernatants using a direct fluorimetric assay (Burke and Mayer 1974) as described previously (Munkittrick et al. 1994).

Statistical analysis

Differences in responses between upstream (reference) and downstream (effluent exposed) white sucker were detected for each

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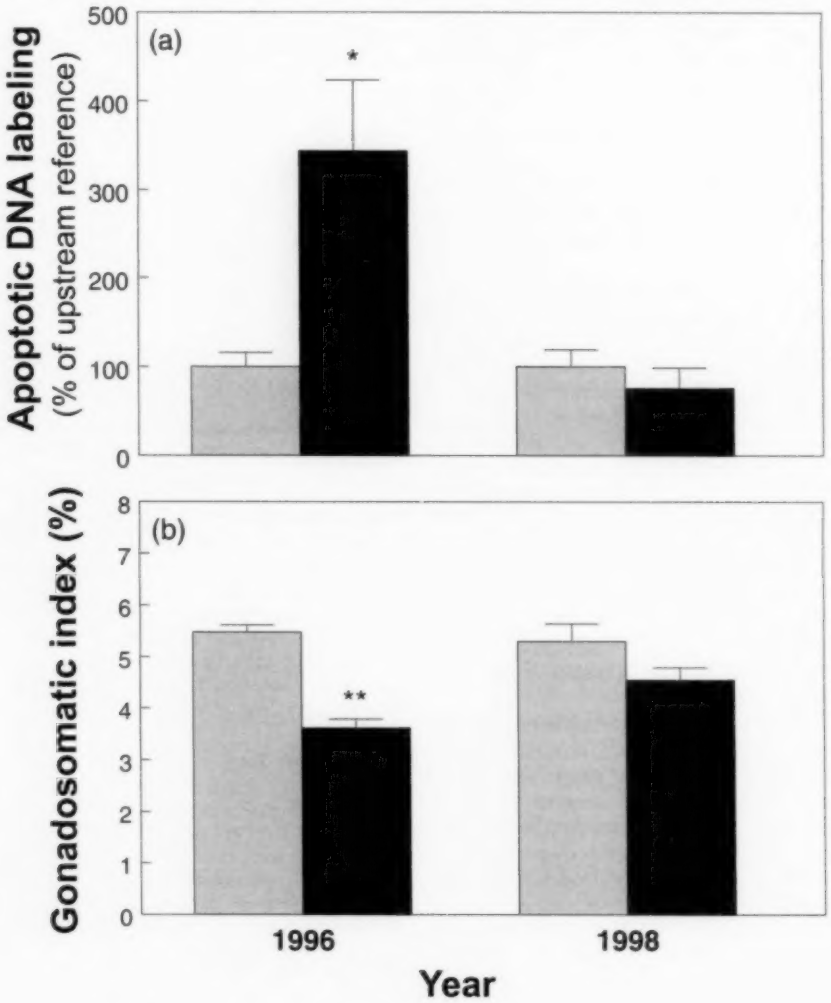
Fig. 1. Relationship between the extent of ovarian cell apoptosis and GSI (ovary to body weight ratio) in vitellogenic female white sucker exposed to bleached pulp mill effluent (solid bars) or collected from an upstream reference site (hatched bars). Fish were collected before (1996) and after (1998) mill maintenance changes aimed at minimizing the release of process chemicals. (*a*) Quantitation of apoptotic DNA fragments (<15 kilobase pairs) in ovarian cells determined using 3′-end labeling. (*b*) GSI. Data are means ± SEM of 6–12 fish. Statistical comparisons were performed between upstream and downstream fish in each year individually. *Significantly different from upstream fish (ANOVA, $p < 0.05$); **significantly different from upstream fish (ANOVA, $p < 0.001$).



year individually (1996 or 1998) using one-way analysis of variance (ANOVA). Differences in ovary weight between sites were detected using analysis of covariance with body weight as covariate. For ovarian cell apoptosis and HSP70 data, comparisons between fish collected upstream and downstream in 1996 or 1998 were expressed as percentages of the upstream reference fish for that year.

Results

Quantification of low molecular weight DNA fragments (<15 kilobase pairs) revealed a 3.4-fold greater extent of ovarian cell apoptosis in vitellogenic white sucker collected 4 km downstream of the bleached kraft pulp mill in 1996 in comparison with fish collected upstream ($p = 0.013$) (Fig. 1*a*). The elevated ovarian cell apoptosis was associated with a significant reduction in GSI in fish collected downstream ($p < 0.001$) (Fig. 1*b*). In fish collected 40 km downstream of the mill in 1996, where effluent concentration was diluted to approximately 1.3% (Munkittrick et al. 1998), there were no differences in GSI (5.90 ± 0.19) and ovarian cell apoptosis (195 ± 24%) compared with fish collected upstream (data not shown). There were no differences in ovarian cell apoptosis or GSI between fish collected upstream and 4 km downstream of the mill in September 1998 (Fig. 1).

The anti-HSP70 antibodies used in this study recognize both the constitutive 73-kilodalton and inducible 72-kilodalton forms of HSP70 (Forsyth et al. 1997). Distinct immunoreactive band doublets at approximately 70 kilodaltons were

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Fig. 2. Ovarian HSP70 expression in vitellogenic female white sucker exposed to bleached pulp mill effluent (solid bars) or collected from an upstream reference site (hatched bars). Fish were collected before (1996) and after (1998) mill maintenance changes aimed at minimizing the release of process chemicals. Ovarian HSP70 expression was quantified using Western blotting and densitometry of immunoreactive bands and expressed as percentages of the upstream reference site. At the top are shown representative Western blots of HSP70. Data are means ± SEM of six fish. Statistical comparisons were performed between upstream and downstream fish in each year individually. *Significantly different from upstream fish (ANOVA, $p < 0.01$).

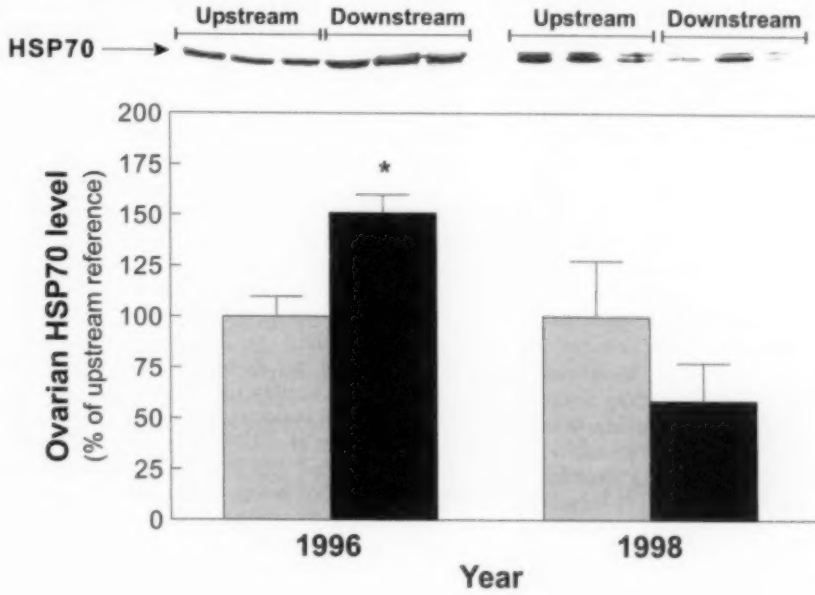

Table 1. Plasma testosterone and 17β-estradiol concentrations and hepatic EROD activities in vitellogenic female white sucker collected upstream and downstream of a bleached kraft pulp mill at Smooth Rock Falls, Ontario, in 1996 and 1998.

	1996		1998	
Parameter	Upstream	Downstream	Upstream	Downstream
Plasma testosterone ($pg \cdot mL^{-1}$)	1226±243	775.0±137	652.0±124	481.0±62.8
Plasma 17β-estradiol ($pg \cdot mL^{-1}$)	2389±165	2119±144	1924±174	1347±111[a]
Hepatic EROD activity ($nmol \cdot min^{-1} \cdot mg\ protein^{-1}$)	2.96±1.14	4.66±0.99[b]	1.58±0.22	3.12±0.52[a]

Note: Data are means ± SEM of 10–15 fish.
[a]Significantly different from upstream fish (ANOVA, $p < 0.01$).
[b]Significantly different from upstream fish (ANOVA, $p < 0.05$).

observed on Western blots, particularly for the 1998 samples that were run on large-format gradient gels. However, we were not able to distinguish between constitutive and inducible forms in this species, and thus, total HSP70 was quantified using densitometry of both immunoreactive bands present at 70 kilodaltons. Ovarian HSP70 expression was significantly elevated in fish collected downstream in 1996 in comparison with fish collected upstream ($p = 0.004$) (Fig. 2). In 1998, there was no significant difference in HSP70 expression between fish collected upstream and downstream of the mill (Fig. 2). Plasma concentrations of testosterone and 17β-estradiol were not significantly different between females collected upstream or downstream of the mill in 1996. Plasma 17β-estradiol but not testosterone levels were lower ($p = 0.007$) in females collected downstream of the mill in 1998 in comparison with fish collected upstream (Table 1). Hepatic EROD activity was higher in females collected downstream of the mill in 1996 ($p = 0.013$) and 1998 ($p = 0.005$) (Table 1) compared with fish collected upstream.

Discussion

Vitellogenic white sucker collected downstream of the bleached kraft pulp mill at Smooth Rock Falls in 1996 had elevated ovarian cell apoptosis, reduced GSI, and increased expression of ovarian HSP70 in comparison with fish collected upstream. These findings were consistent with the results of a previous study showing that preovulatory white sucker collected downstream of a bleached kraft mill at Terrace Bay exhibited a 10-fold higher extent of ovarian cell apoptosis than fish collected from an ecologically similar reference site (Janz et al. 1997). Similar to the present study, the increased ovarian cell apoptosis was associated with reduced GSI and increased HSP70 expression (Janz et al.

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1997). In contrast with these findings, in the present study, there were no differences in GSI, ovarian cell apoptosis, or HSP70 expression between fish collected upstream and downstream of the mill in 1998 following a number of mill alterations. Overall, these data suggest a consistent inverse relationship between ovarian size and the extent of ovarian somatic cell apoptosis in fish chronically exposed to pulp mill effluents. Although germ cells also undergo apoptosis (Hsueh et al. 1994), the apoptotic DNA fragmentation determined in this study was due predominantly to granulosa and theca cell death, since these cells represent the majority of DNA isolated from vitellogenic follicles (Janz and Van Der Kraak 1997). These results support the hypotheses that certain components of BKME directly or indirectly stimulate apoptotic cell death in ovarian follicular cells, that this response occurs at different stages of ovarian development, and that such elevations in apoptosis may represent a key mechanism responsible for reductions in ovary size, fecundity, and egg size previously observed in female fish chronically exposed to pulp mill effluents.

It is not known whether increased expression of ovarian HSP70 in effluent-exposed fish is mechanistically linked to elevated apoptosis or represents a general response to environmental stress. HSP70 is a family of highly abundant and evolutionarily conserved proteins that include a constitutive 73-kilodalton form (hsc70) and an inducible 72-kilodalton form (hsp72) in most cells (reviewed in Feder and Hofman 1999; Mallouk et al. 1999). The function of constitutive and inducible forms of HSP70 is similar and involves refolding of improperly folded proteins or chaperoning irreversibly misfolded proteins for degradation. Recent studies in mammals have shown that both constitutive and inducible forms of HSP70 play pivotal roles in protecting cells from stress-induced apoptosis (Mosser et al. 1997; reviewed in Mallouk et al. 1999). Thus, in 1996, ovarian HSP70 expression may have increased as a protective cellular response to BKME-stimulated ovarian cell apoptosis. This conclusion is supported by similar results in a previous study in preovulatory white sucker exposed to BKME (Janz et al. 1997). In addition, vitellogenic white sucker collected upstream and downstream of a thermomechanical pulp mill in Kapuskasing, Ontario, in 1996 exhibited a statistically similar extent of ovarian cell apoptosis, HSP70 expression, and GSI (data not shown), suggesting that certain components of BKME that are absent from thermomechanical mill effluents may be responsible for the observed ovarian effects. Further studies are necessary to better characterize the HSP70s involved and investigate their roles in normal cellular homeostasis, the stress response, and apoptosis in reproductive tissues of fishes.

Although reductions in circulating levels of sex steroid hormones are still observed in fish exposed to certain pulp mill effluents, there have been no consistent differences in plasma steroid levels between female white sucker collected upstream or downstream of the mill at Smooth Rock Falls since secondary treatment was initiated in 1994 (Munkittrick et al. 1998). It does not appear that alterations in sex steroid hormones were responsible for the reduced GSI and elevated ovarian apoptosis observed at this mill in 1996. Similar to higher vertebrates (Hsueh et al. 1994), ovarian cell apoptosis is regulated by the integrative effects of multiple hormones and growth factors in fish (Janz and Van Der Kraak 1997; Van Der Kraak et al. 1997). It is unlikely that decreased plasma levels of a single survival factor such as 17β-estradiol would explain elevated apoptosis. This conclusion is supported by the present study, since plasma 17β-estradiol levels were lower in fish collected downstream in 1998, although there was no difference in ovarian cell apoptosis. Furthermore, a previous study reported elevated ovarian cell apoptosis in fish exposed to BKME that was associated with increased 17 β-estradiol and reduced testosterone levels (Janz et al. 1997). Although increased apoptosis of the steroid-producing granulosa and theca cells might be expected to result in reductions in circulating steroid levels, such hormones are regulated by negative feedback control of gonadotropin release from the pituitary gland. Thus, increased release of gonadotropins may be able to counteract the effect of smaller populations of granulosa and theca cells due to apoptosis. Overall, it appears that alterations in circulating sex steroids were not causally related to increased ovarian cell apoptosis.

Recovery of hepatic detoxification enzymes such as EROD has also occurred in fish downstream of many mills (Munkittrick et al. 1998), although in the present study, hepatic EROD activity remained slightly induced (approximately twofold) in both years. The potential relationship between induction of EROD activity and increases in ovarian cell apoptosis and HSP70 expression in fish is unclear. Induction of EROD activity is a specific catalytic marker of cytochrome P4501A (CYP1A) protein expression. CYP1A is a highly evolutionarily conserved protein expressed following exposure to environmental toxicants such as polycyclic aromatic hydrocarbons and chlorinated hydrocarbons that activate AhR. Increasing evidence in mammals suggests that both AhR and CYP1A have important roles in normal growth and development, particularly in reproductive tissues (Nebert et al. 2000). A connection between normal AhR function and apoptosis is emerging, particularly in atresia of primary oocytes and developing ovarian follicles of mammals (Robles et al. 2000). A correlation between AhR stimulation, CYP1A expression, and increased apoptosis has also been reported in Japanese medaka (*Oryzias latipes*) embryos (Cantrell et al. 1998). However, in the present study, the similar low degree of EROD induction in 1996 and 1998 does not suggest a causal role for AhR in the elevated apoptosis observed only in 1996.

In summary, this study provides further evidence that certain chemicals present in BKME stimulate apoptotic cell death and HSP70 expression in the ovary of fish and that these cellular responses may be mechanistically related to reductions in ovary size in fish exposed chronically to mill effluents. Although the specific effluent components that were reduced or eliminated following the mill maintenance changes are not known, the changes resulted in significant recovery of these responses in comparison with 1996. Further research is needed to examine the potential linkages between cellular responses (e.g., apoptosis, HSP70 expression, steroidogenesis) and integrated responses at higher levels of biological organization (e.g., atresia, fecundity, ovary size).

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PERSPECTIVE

Permutation tests for univariate or multivariate analysis of variance and regression

Marti J. Anderson

Abstract: The most appropriate strategy to be used to create a permutation distribution for tests of individual terms in complex experimental designs is currently unclear. There are often many possibilities, including restricted permutation or permutation of some form of residuals. This paper provides a summary of recent empirical and theoretical results concerning available methods and gives recommendations for their use in univariate and multivariate applications. The focus of the paper is on complex designs in analysis of variance and multiple regression (i.e., linear models). The assumption of exchangeability required for a permutation test is assured by random allocation of treatments to units in experimental work. For observational data, exchangeability is tantamount to the assumption of independent and identically distributed errors under a null hypothesis. For partial regression, the method of permutation of residuals under a reduced model has been shown to provide the best test. For analysis of variance, one must first identify exchangeable units by considering expected mean squares. Then, one may generally produce either (*i*) an exact test by restricting permutations or (*ii*) an approximate test by permuting raw data or some form of residuals. The latter can provide a more powerful test in many situations.

Résumé : La stratégie la plus appropriée pour générer une distribution de permutation en vue de tester les termes individuels d'un plan expérimental complexe n'est pas évidente à l'heure actuelle. Il y a souvent plusieurs options, dont la permutation restreinte et la permutation d'une quelconque forme des résiduels. On trouvera ici un résumé d'informations récentes empiriques et théoriques sur les méthodes disponibles, ainsi que des recommandations pour leur utilisation dans des applications unidimensionnelles et multidimensionnelles. L'emphase est mise sur les plans complexes d'analyse de variance et de régression multiple (i.e. les modèles linéaires). Dans un travail expérimental, la supposition d'échangeabilité requise pour un test par permutation est assurée par l'assignation au hasard à des unités des divers traitements. Dans le cas d'observations, l'échangeabilité équivaut à supposer que les erreurs, dans une hypothèse nulle, sont indépendantes et distribuées de façon identique. Pour la régression partielle, la méthode de permutation des résiduels dans un modèle réduit s'est avérée la meilleure. Pour l'analyse de variance, il faut d'abord identifier les unités échangeables à l'examen des carrés moyens attendus. Ensuite, il est généralement possible de produire (*i*) un test exact en restreignant les permutations ou alors (*ii*) un test approximatif en permutant les données brutes ou une forme quelconque des résiduels. Cette dernière méthode fournit, dans plusieurs situations, un test plus puissant.

[Traduit par la Rédaction]

Introduction

Biologists and ecologists are faced with increasingly complex circumstances for the statistical analysis of data. In experimental and observational studies, the assumptions that errors are independent and identically distributed as normal random variables with common variance and an expectation of zero, as required by traditional statistical methods, are no longer generally considered realistic in many practical situations (e.g., Clarke 1993; Gaston and McArdle 1994; Anderson 2001). The traditional approach relies on the assumptions to use the statistical distribution of a test statistic, such as t, χ^2, or F, that is known under a specified null hypothesis, for the calculation of a probability (i.e., a P value), commonly relying on tabulated values. An alternative to this traditional approach that does not rely on such strict assumptions is to use a permutation test. A permutation test calculates the probability of getting a value equal to or more extreme than an observed value of a test statistic under a specified null hypothesis by recalculating the test statistic after random re-orderings (shuffling) of the data.

The first descriptions of permutation (also called randomization) tests for linear statistical models (including analysis of variance and regression) can be traced back to the early half of this century in the work of Neyman (1923), Fisher

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(1935), and Pitman (1937*a*, 1937*b*, and 1937*c*). Such tests are computationally intensive, however, and the use of these tests as opposed to the traditional normal-theory tests did not receive much attention in the natural and behavioral sciences until much later, with the emergence of widely accessible computer power (Edgington 1995; Manly 1997; Good 2000).

There is general agreement concerning an appropriate method of permutation for exact tests of hypotheses in one-way analysis of variance (ANOVA) or simple linear regression (or, more simply, tests for the relationship between two variables, e.g., Edgington (1995) and Manly (1997)). This is not the case, however, for tests of individual terms in the context of multiple linear regression (e.g., Kennedy and Cade 1996; Manly 1997) or multifactorial analysis of variance (ter Braak 1992; Edgington 1995; Gonzalez and Manly 1998). Thus, how to do permutations for complex experimental designs is not at all clear. Such complex designs are common, however, in biological and ecological studies, where, usually, several factors are of interest, concomitant environmental variables are measured, or nested hierarchies of sampling at different temporal and spatial scales are necessary.

Recent work in this area has resolved many of these arguments, demonstrating the differences (and (or) similarities) among various approaches, both theoretically (Anderson and Robinson 2001) and empirically (Gonzalez and Manly 1998; Anderson and Legendre 1999; M.J. Anderson and C.J.F. ter Braak, unpublished data). However, a unified treatment of the subject that provides an accessible summary of these recent results and indicates how and when different methods should be used does not currently exist. The purpose of this paper is, therefore, to review and consolidate recent findings and to provide some practical and accessible recommendations for ecologists to construct permutation tests in regression, multiple regression, and analysis of variance, for univariate or multivariate response data.

With the rediscovery and increasing use of permutation tests, praised for being "distribution free," there has sometimes been a failure to recognize assumptions still inherent in these tests for different contexts and statistical inferences. This paper begins, therefore, with a review of the origin and history of permutation tests, clarifying and describing their assumptions in terms of where their validity for statistical inference lies in different contexts. This is followed by a description of the exact permutation test for simple designs, namely, one-way ANOVA and simple linear regression, and a summary of the important considerations for these tests on multivariate response data. Ensuing sections deal in detail with constructing tests for complex designs, with practical recommendations concluding the paper.

Background and rationale for permutation tests

Experimental tests: validity through random allocation

A clear description of a statistical test using permutations of the original observations occurs in the book *Design of Experiments* by R.A. Fisher (1935). Fisher described an experiment by Darwin, first analyzed by Galton, to test the null hypothesis of no difference in the growth rates of *Zea mays* (maize) plants that were self-fertilized versus those that were cross-fertilized. Fisher justifies the validity of the statistical test by virtue of the randomization procedure that is carried out by the experimenter at the beginning of the experiment.

Suppose pairs of seeds, with each pair containing one self- and one cross-fertilized seed (designated, say, by the labels A and B, respectively), are randomly assigned to pairs of plots (in Darwin's experiment there were $n = 15$ such pairs). The determination of which kind of seed will be allocated to which plot in each pair is determined by a random process, such as the flip of a coin. The experimenter then plants the seeds accordingly and, after a period, measures the growth of the plants in each pair (i.e., y_{Ai} and y_{Bi} for each of $i = 1,\ldots,n$ pairs). (Fisher (1935) noted with dismay that such a random allocation procedure was, unfortunately, not in fact carried out by Darwin in his study).

Consider the sum of differences in the measurements obtained between the plants in each pair of plots ($D_{obs} = \Sigma_{i=1}^{n} d_i$, where $d_i = (y_{Ai} - y_{Bi})$ and $i = 1,\ldots,n$). Consider also the null hypothesis H_0: the two series of seeds are random samples from the same population. Under H_0, the identity of the seeds randomly allocated to plots could have resulted in d_i for any pair being either positive or negative. In particular, what is "randomized" for the experiment a priori—the allocation of seed types in a particular order (A,B) or (B,A) on pairs of plots—is the same thing that can be randomized to realize all the alternative allocations (and associated outcomes) we could have obtained if H_0 were true. If we consider all possible 2^{15} realizations of the experiment consisting of all possible allocations of the seed pairs to plots, we obtain a distribution of D under the null hypothesis. By comparing the observed value D_{obs} with the distribution of D for all possible outcomes under H_0, we can calculate the probability under a true null hypothesis associated with the particular value we obtained: P = the number of values of $|D| \geq D_{obs}$, divided by the total possible number of values of $|D|$.

This probability is only conditional on the absolute values of the observations (i.e., on the values obtained, not on their sign) and the random allocation of the seeds (A or B) to experimental units (plots). It is the experimental design that is considered random, while the observed values and their associated errors are considered fixed.

Note that the procedure of random allocation at the outset is what ensures the validity of the test. By validity, I mean that the probability of rejecting the null hypothesis when it is true is exactly the level of significance (α) chosen for the test a priori. The test is exact. No assumption concerning the nature of the distribution of errors associated with seeds (or measured outcomes) is necessary. Fisher's view of the validity of the permutation test, as for the familiar normal-theory tests, derived from randomization in experimental design (Fisher 1935, p. 51).

Fisher's idea that the random allocation of treatments in an experimental design ensures the validity of a test by permutation has been adopted and re-iterated by many authors throughout this century (e.g., Scheffé 1943; Kempthorne 1955; Still and White 1981). Kempthorne (1955) gave a useful summary and notation for the idea that the random component in a linear model for a permutation test is the design component, while the errors and observations are treated as fixed.

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Not long after Fisher, the notion of a test by permutation was championed in a series of papers by Pitman (1937*a*, 1937*b*, and 1937*c*), who particularly emphasized that the tests were valid because they did not make reference to an unknown population. For purposes of the test, we consider that the sample we have obtained *is* the population of interest. This, he claimed, meant that the test of significance had no distributional assumptions. Kempthorne (1955) and Edgington (1995) have especially popularized Pitman's idea that statistical inferences from randomization tests may not be regarded as extending beyond the sample itself, except when the extra assumption of random sampling from a population holds.

Thus, for statistical inference in an experimental study using permutation of the observations, the only real requirement for the test is that the units have received a random allocation of experimental treatments. For this approach, we consider only the null hypothesis H_0: the treatments have no effect on the units. Together with the initial random allocation, this provides for exchangeability of units without reference to independence or to any distribution. Our inferences are restricted, in this case, to the result in terms of the sample itself. If we intend to make inferences from our sample to a wider population, then an added assumption is necessary, namely, any effect of treatments on the observed units is the same as the effect of treatments on the set of units in a wider specified population. Such an assumption is weaker than the assumption that we actually have a random sample from the population.

As an added note, it is known that, when the assumptions of the normal-theory test are fulfilled, the permutation test and normal-theory test converge (e.g., Fisher 1936). Also, the related approach of bootstrapping provides a test that is asymptotically equivalent to the permutation test (Romano 1988).

Observational tests: validity through exchangeability

The validity of the permutation test does not have the same origin for studies that are observational rather than experimental (e.g., Kempthorne 1966). By an observational study, I mean that the experimenter does not allocate experimental treatments to the units studied, but that a classification of units already exists about which one wishes to test hypotheses. For example, we may wish to test the null hypothesis H_0: there is no difference in the numbers of snails occurring in a midshore region and a high-shore region of a rocky intertidal shore. We can sample the numbers of snails in random representative units (such as quadrats) from each of these areas, but there is no way in which we may "allocate" the classification of being in a high-shore or midshore area to the units randomly. (The important issue of pseudoreplication in observational (mensurative) or experimental ecological field studies (Hurlbert 1984) is not treated in this simplified example.)

In such a situation, we must consider a conceptual notion of *exchangeability* among the units under H_0. If the numbers of snails in the high-shore and midshore areas are really not different, then the values obtained in quadrats labeled "high shore" and quadrats labeled "midshore" are exchangeable. Such units are certainly not exchangeable by virtue of the design, as would have been the case if we somehow could have experimentally randomly "allocated" the quality of being in either the midshore or the high-shore region. This classification exists in nature without our having experimental control over it. For observational data, we must assume exchangeability under H_0.

The assumption of exchangeability under H_0 is equivalent to the assumption of independent and identically distributed (iid) random errors. We do not need to assume *what kind* of population distribution our particular errors are obtained from (normal or otherwise), only that whatever that population is, the errors associated with the units we have are iid. Note that this means that a test by permutation does not avoid the assumption of homogeneity of error variances—the observation units must be exchangeable under the null hypothesis.

It is a common misconception that a permutation test has "no assumptions." However, exchangeability must either be assumed (e.g., for observational data) or it must be ensured by virtue of an a priori random allocation of units in an experiment. The reliance of the permutation test on exchangeable units has been clearly shown in empirical studies (Boik 1987; Hayes 1996). For example, a test using the t statistic for a difference in mean between two samples will be sensitive to differences in error variances, even if the P value is calculated using permutations (Boik 1987).

Although Kempthorne and Doerfler (1969) suggested distinguishing tests on observational data from experimental tests by calling them "permutation tests" and "randomization tests," respectively, I follow Manly (1997) in choosing not to make this distinction. It is the philosophical context, sampling design, and subsequent inferences that are distinguishable in these two cases, rather than any physical difference in the way the test itself is done. Note also, in this context, that, if we have a manipulative experimental design where random allocation did not form part of the procedure, then the iid assumption for the test by permutation still applies.

In what follows, I consider that the permutation tests discussed will assume iid random errors (not necessarily normal) that are exchangeable under the null hypothesis, as this provides for the most general application of ideas to either experimental or observational studies. This assumption can be relaxed to a more general assumption of experimental exchangeability under the null hypothesis (allowing the assumptions of independence and homogeneity to be ignored), if this is ensured by physical random allocation in the experimental design a priori.

Simple designs

One-way univariate ANOVA

Consider a one-way ANOVA design with n replicate observations (measurements of a response variable) from each of k groups. A reasonable statistic to test for differences among the groups is Fisher's F statistic. Suppose the null hypothesis is true and the groups are not really different (in terms of the measured variable). If this is the case, then the observations are exchangeable between the different groups. That is, the labels that are associated with particular values, identifying them as belonging to a particular group, could be randomly shuffled (permuted) and a new value of F could be obtained, which we will call, say, F^{π}. If we were to calculate

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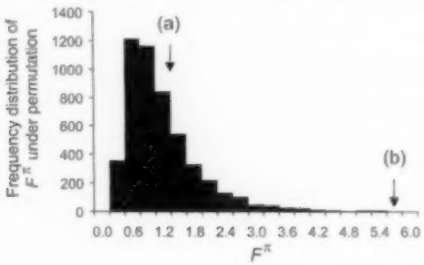
Fig. 1. Frequency distribution of values of the F statistic (F^{π}) for 5000 permutations of a data set with $n = 10$ replicates in each of three groups. Depending on the observed value of the F statistic obtained from the original data (F_{obs}) by reference to this distribution, the null hypothesis of no differences among groups may be accepted (where $F_{obs} < F_{crit}$) (*a*) or rejected (where $F_{obs} \geq F_{crit}$) (*b*), where α is the chosen significance level and F_{crit} is the value of F that is equal to or exceeded by $100\alpha\%$ of the values of F^{π} obtained by permutation.



F^{π} for all the different possible allocations of the labels to the observed values, this would give the entire distribution of the F statistic under a true null hypothesis, given the particular data set (Fig. 1).

To calculate a P value for the test, we compare the value of F calculated on the original data with the distribution of values F^{π} obtained for a true null by permuting the labels (Fig. 1). The empirical frequency distribution of F^{π} can be articulated entirely: that is, the number of possible ways that the data could have been re-ordered is finite. The probability associated with the null hypothesis is calculated as the proportion of the F^{π} greater than or equal to F. Thus,

$$(1) \qquad P = \frac{(\text{no. of } F^{\pi} \geq F)}{(\text{total no. of } F^{\pi})}$$

In this calculation, the observed value is included as a member of the distribution. This is because one of the possible random orderings of the treatment labels is the ordering that was actually obtained. This P value gives an exact test of the null hypothesis of no differences among groups, that is, the rate of Type I error is exactly equal to the significance level chosen for the test a priori (Hoeffding 1952).

The usual scientific convention of an a priori significance level of $\alpha = 0.05$ is generally used to interpret the significance of the result, as in other statistical tests. The P value can also be viewed as providing a level of confidence with which any particular opinion about the null hypothesis may be held (e.g., Fisher 1955; Freedman and Lane 1983). The only assumption of the permutation test is that the observations are exchangeable under a true null hypothesis.

With k groups and n replicates per group, the number of distinct possible outcomes for the F statistic in a one-way test is $(kn)!/[k!(n!)^k]$ (e.g., Clarke 1993). In reality, it is usually not practical to calculate all possible permutations, because with modest increases in n this becomes prohibitively time-consuming. A P value can also be obtained by taking a large random subset of all possible permutations to create the distribution (Hope 1968). Increasing the number of permutations increases the precision of the P value. Manly (1997) suggested using at least 1000 permutations for tests with an α level of 0.05 and at least 5000 permutations for tests with an α level of 0.01.

Sometimes the total number of permutations is greatly reduced in exact permutation tests for terms in complex ANOVA designs. This occurs when permutations are restricted to occur only within categories of other factors. If, for example, there are only 20 possible permutations, then the smallest P value that can be obtained is 0.05. Although such permutation tests still have exact Type I error, their power is extremely low (M.J. Anderson and C.J.F. ter Braak, unpublished data). If the total possible number of permutations is less than 100 for the exact test, it would be a good idea to consider using an approximate permutation test (as described in Partial regression and Factorial (orthogonal) designs, below).

The one-way multivariate case

Consider the one-way analysis of a multivariate set of measurements on p variables (e.g., species) for each of the n replicate observations in each of the k groups. Let the data be organised so that the nk observations are rows and the p variables are columns in a matrix. For the multivariate test of differences among groups by permutation (e.g., using the R statistic of Clarke 1993), the assumption of exchangeability applies to observations (rows) not variables (columns). Permutation in the multivariate context means randomizing whole rows (Fig. 2*a*). The numbers in the raw data matrix are not shuffled just anywhere. An entire row of values is shuffled as a unit. This is important for two reasons. First, the units that are exchangeable under the null hypothesis of no treatment effect are the replicate observations, not the numbers of individual species in each observation. Second, in multivariate analysis, the species will probably have some non-zero correlation, that is, some relationship with one another. They are not likely to be independent, so they cannot be considered to be exchangeable in the sense of a permutation test.

If the multivariate analysis is based on a distance matrix (i.e., a measure of dissimilarity or distance calculated between every pair of values, as in the method of Clarke (1993)), then the permutation of observations can be achieved by permuting the rows and columns of the distance matrix simultaneously (Fig. 2*b*). As an example, consider a new ordering of six rows of an original data matrix from {1, 2, 3, 4, 5, 6}, the original ordering, to {6, 2, 4, 3, 1, 5}, a new ordering under permutation. One does not need to recalculate the (6×6) distance matrix, because distances (or dissimilarities) between pairs of observations have not changed with the permutation. What has changed is the ordering of the observations (i.e., their labels). In other words, the observations are exchangeable, not the distances. So the new distance matrix under this particular permutation has simultaneously re-ordered rows as {6, 2, 4, 3, 1, 5} and columns as {6, 2, 4, 3, 1, 5}.

A further consideration for permutation tests with multivariate data is that the assumption of iid observations (exchangeability) means that the test will be sensitive to differences in the multivariate dispersions of observations (e.g., Clarke 1993). Let each observation be a point in the space of p dimensions (variables or axes). Variability in the values taken along any of these axes corresponds to the dispersion

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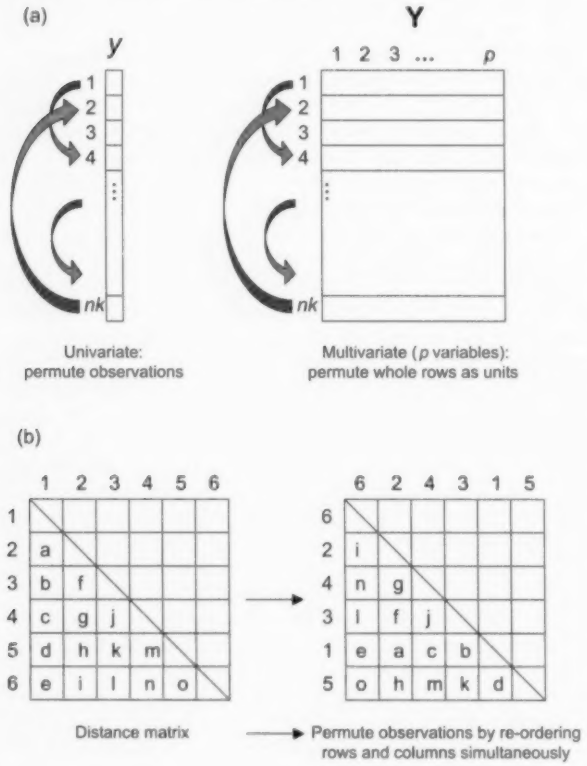
Fig. 2. (*a*) Schematic representation of the relationship between permutation tests for univariate versus multivariate data. In each case, the exchangeable units are the observations but, for multivariate data, the observations are whole rows of information for *p* variables. (*b*) Schematic representation of the permutation of multivariate observations done directly on the distance matrix. The numbers correspond to the original sampling units (rows and columns) and the letters correspond to distances between pairs of units.



of points in multivariate space. Just as a univariate test by permutation will be sensitive to deviations from the assumption of homogeneity of variances (Boik 1987; Hayes 1996), so too will a multivariate test be sensitive to deviations from the assumption that the observations in different groups have similar dispersions (Clarke 1993; Anderson 2001). The interpretation of significant results must be treated with caution in this regard.

Equivalent test statistics under permutation

Some researchers have highlighted that simplified test statistics can give equivalent *P* values under permutation, thus saving computational time (Edgington 1995; Manly 1997). Simplification of the test statistic is done by considering components of the calculation that can be dropped because they remain the same for all permutations or by considering a simpler test statistic that is monotonically related to the original test statistic.

For example, in the case of the univariate one-way *F* ratio, the degrees of freedom do not change, no matter what the ordering of the data is, nor does the total sum of squares. Thus, either the among-group or within-group sum of squares (SS_A or SS_W, respectively) is monotonically related to the *F* ratio. This means that the position of the observed value of SS_A relative to the distribution of SS_A^{π} under permutation is the same as that of the observed value of *F* relative to the distribution of F^{π}. SS_A can therefore be used as an equivalent (and more simply calculated) statistic to obtain a *P* value.

In cases of more complex designs, the idea of an equivalent test statistic under permutation becomes less useful. In many cases, it is not possible to simplify the test statistic much beyond what is called a "pivotal" statistic. A pivotal statistic is defined as follows: in general, to find a confidence interval for a parameter θ, based on an estimator $T(\mathbf{y})$, where $\mathbf{y}$ denotes a vector of *n* independent random variables, a pivotal statistic τ has the following properties: (*i*) τ is a function of $T(\mathbf{y})$ and θ, (*ii*) τ is monotonic in θ, and (*iii*) τ has a known sampling distribution that does not depend on θ or on any other unknown parameters. Statistics like *t*, *F*, and the correlation coefficient, *r*, (or its square) are pivotal. A slope coefficient (in regression) or a sum of squares or mean square (in ANOVA) is not pivotal, because each depends on the value of some unknown parameter(s) for any particular data set, even if the null hypothesis is true. Approximate permutation tests in complex designs, described in more detail below, generally do not work, if a nonpivotal statistic is used (Anderson and Legendre 1999). In practice, it is always wise to use a pivotal statistic for a permutation test, especially for complex designs. Statistics like *t*, *F*, or r^2 also have the advantage of being interpretable in themselves: their value has some meaning that can be compared across different studies.

Simple linear regression

Consider *n* pairs of observations of a random variable *Y* with fixed values of a variable *X* collected to test the null hypothesis of no (linear) relationship between *Y* and *X*. I restrict attention to the case of Model 1 regression (sensu Sokal and Rohlf 1981). Given the linear model of $Y = \mu + \beta X + \varepsilon$, the null hypothesis is that the slope $\beta = 0$. An appropriate test statistic for the two-tailed test is the square of the least-squares correlation coefficient, r^2.

The rationale for the permutation test for simple linear regression follows the same general rationale as that for the one-way ANOVA case. Namely, if the null hypothesis of no relationship between the two variables is true, then the *n* observations of *Y* could have been observed in any order with respect to the *n* fixed values of *X*. An exact test is therefore given by recalculating the test statistic (called, say, $(r^2)^{\pi}$) for each of the possible re-orderings (permutations) of *Y*, with the order of values of *X* remaining fixed. The probability for the test is obtained by comparing the original observed value r^2 with the distribution of values of $(r^2)^{\pi}$ obtained for all permutations. The *P* value is that fraction of the permutations for which $(r^2)^{\pi} \geq r^2$.

In this case, there are *n*! unique possible permutations, which clearly gets to be very large with moderate increases in *n*. In practical terms, the same considerations are applied as for ANOVA: a random subset of all possible permutations is obtained and the *P* value calculated as in eq. 1, but for r^2. Note that the only assumption of the test is that the errors are iid or, more generally, under a true null hypothesis, the

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observations Y are exchangeable. The errors do not have to be normally distributed. In a similar fashion to the ANOVA case, the assumption of exchangeability is assured if the n units were randomly allocated to each of the values of X at the beginning of the experiment.

Complex designs—multiple and partial regression

Multiple regression

Let Y be a biological or ecological response variable (like the growth of bivalves) and X and Z be some independent variables (such as particulate organic matter (POM) and temperature, respectively) predicted under some hypothesis to affect Y. Say that the growth of bivalves is measured in each of n different combinations of POM (Z) and temperature (X), where these have either been fixed experimentally or, more generally, are simply measured in situ along with Y. The linear model is

$$(2) \qquad Y = \beta_0 + \beta_1 X + \beta_2 Z + \varepsilon'$$

If X and Z were measured with error, this is not strictly a Model I regression. Data can, however, be analyzed as a fixed case (Model I), provided the tests for significant relationships among the variables are interpreted as conditional on the values of X and Z actually observed (e.g., see Neter et al. 1996 for details).

To test for the relationship of Y versus X and Z together, the null hypothesis is H_0: $\beta_1 = \beta_2 = 0$. An appropriate test statistic is the coefficient of multiple determination, R^2 (e.g., Sokal and Rohlf 1981; Neter et al. 1996). To do the test by permutation requires careful consideration of what is exchangeable under the null hypothesis. If H_0 is true, the model becomes $Y = \beta_0 + \varepsilon'$. So, under the assumption that the errors are iid, the observations (Y) are exchangeable under the null hypothesis. That is, if Y has no relationship with X and Z together, the values obtained for Y could have been observed in any order relative to the fixed pairs of values of X and Z. So, an exact P value for the test in multiple regression can be obtained by randomly permuting Y, leaving X and Z fixed, and recalculating $(R^2)^\pi$ under permutation, as was done for simple linear regression.

Partial regression

When several independent variables are measured (or manipulated), the researcher is usually interested in something more specific than the general test of multiple determination. For the example involving bivalves, the purpose may be to investigate the relationship between the growth of bivalves and POM, given any effects of temperature. The null hypothesis is H_0: $\beta_2 = 0$. The hypothesis is that POM explains a significant proportion of the variability in bivalve growth over and above any effect of temperature. The effect of temperature (if it is there) must be "removed" to allow a test for any relationship between growth and POM. This is a partial regression with temperature as a covariable.

An appropriate test statistic for the relationship between Y and Z given X is the squared partial correlation coefficient

$$(3) \qquad r^2_{YZ.X} = \frac{(r_{YZ} - r_{XZ}\, r_{YX})^2}{(1 - r_{XZ})^2 (1 - r_{YX})^2}$$

where r_{YZ} is the simple correlation coefficient between Y and Z, r_{XZ} is that between X and Z, and so on. For a permutation test in this situation, consider what the model would be if the null hypothesis were true:

$$(4) \qquad Y = \beta_0 + \beta X + \varepsilon$$

where β is the simple regression coefficient of Y versus X. Under the assumption of iid errors, what are exchangeable under the null hypothesis are not the observations Y, but rather the errors ε after removing the effect of X. That is, the exchangeable units under H_0 are $(Y - \beta_0 - \beta X)$. These are the units that should be shuffled for the exact permutation test. Unfortunately, the parameters β_0 and β are not known. Thus, for the test of a partial regression (e.g., a test of the relationship between Y and Z given X), no exact permutation test is possible (Anderson and Robinson 2001).

Several approximate permutation methods for this situation have been suggested (e.g., Freedman and Lane 1983; ter Braak 1992; Manly 1997). These methods (Fig. 3) have been compared theoretically (Anderson and Robinson 2001) and in empirical simulations (Anderson and Legendre 1999).

The idea of Freedman and Lane (1983; Fig. 3*b*) is the most intuitively appealing. The essence of their idea is this: although β_0 and β are unknown, they can be estimated by the intercept (b_0) and the regression coefficient (b) from the simple linear regression of Y versus X. The residuals of this regression $R_{Y.X} = (Y - b_0 - bX)$ approximate the errors (ε) that are exchangeable under the null hypothesis. That is, the residuals of the regression of bivalve growth versus temperature can represent the observations after "removing" the influence of temperature. These are exchangeable against fixed values of POM (Z) under the null hypothesis and are, therefore, permuted. For each of the $n!$ possible re-orderings, the value of the squared partial correlation coefficient $(r^2_F)^\pi$ is calculated for the permuted residuals $R^\pi_{Y.X}$ versus Z given X (the subscript F indicates the Freedman and Lane (1983) method). Note that X and Z are not shuffled but remain in their original order. This ensures that the relationship (if any) between X and Z remains intact and does not affect the test for a partial effect of Z on Y given X (e.g., Anderson and Legendre 1999; Anderson and Robinson 2001). The P value is calculated as the proportion of values $(r^2_F)^\pi$ that equal or exceed the value of $r^2_{YZ.X}$ for the original data. This method of permutation is sometimes called permutation "under the null model" or "under the reduced model" (see Anderson and Legendre 1999). Another important aspect of this method to note is that it introduces a further assumption for this particular permutation test: that is, the relationship between Y (bivalve growth) and X (temperature, the covariable) conforms to a linear model. That is, the effect of temperature has only been "removed" in terms of its linear effect on Y.

This permutation test is not exact, but it is asymptotically exact. An asymptotically exact test has a Type I error (the probability of rejecting the null hypothesis when it is true) that asymptotically approaches the significance level chosen for the test (e.g., $\alpha = 0.05$), with increases in n. The larger the sample size, n, the closer the estimate b will be to the

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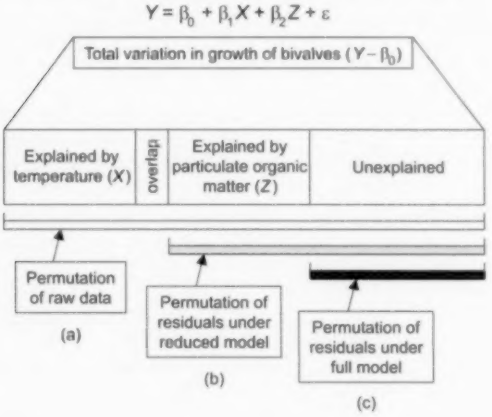
$Y = \beta_0 + \beta_1 X + \beta_2 Z + \varepsilon$
Total variation in growth of bivalves ($Y - \beta_0$)
Explained by temperature (X)
overlap
Explained by particulate organic matter (Z)
Unexplained
Permutation of raw data
(a)
Permutation of residuals under reduced model
(b)
Permutation of residuals under full model
(c)

Fig. 3. Diagram of the partitioning of variability of a response variable (Y, bivalve growth) in terms of two independent variables (temperature, a covariable X, and particulate organic matter, Z). The three methods of approximate permutation for a test of the partial regression of Y on Z given X correspond to permutation of different portions of the variability in the response variable.

true parameter β, so the better the residuals $R_{Y.X}$ will be at estimating the exchangeable errors, ε. Anderson and Robinson (2001) have shown that this method (Freedman and Lane 1983) comes the closest to a conceptually exact permutation test and Anderson and Legendre (1999) have shown that it gives the best empirical results, in terms of Type I error and power, compared with other methods.

One alternative method is to permute the raw observations, Y, while keeping X and Z fixed (Fig. 3*a*; see Manly 1997). The partial correlation coefficient is then recalculated for each permutation. The essential requirement for this to work is that the distribution of the observations (Y) must be similar to the distribution of the errors (ε) under the null hypothesis with a fixed effect of X (Kennedy and Cade 1996; Anderson and Legendre 1999). This may not be true if there is an outlier in X, the covariable. For example, consider that one of the values of temperature (X) is very high relative to the others. Consider also that temperature does affect bivalve growth (say, $\beta = 1.0$), so that the growth of bivalves for that particular temperature is quite large relative to the others. Let the null hypothesis of no effect of POM on growth be true and say the errors follow a normal distribution. Since $Y = \beta_0 + \beta X + \varepsilon$, Y is not distributed like ε, because that one value of X is large relative to the others. When the Y are permuted, the outlying value of Y will no longer be paired with the outlier in X, so the permutation test starts to go awry. The units being shuffled (Y) are not distributed like the exchangeable units under the null hypothesis (ε), so permuting Y does not give an accurate approximate test of H_0.

Kennedy and Cade (1996) and Anderson and Legendre (1999) have shown that the presence of an outlier in the covariable destabilizes this test, often leading to inflated Type I error, so shuffling raw observations is not a method that should generally be used for tests of partial correlation. If, however, the sample size is quite small (i.e., $n < 10$), this method avoids having to calculate residuals as estimates of errors (unlike the method of Freedman and Lane (1983)) and so it may be used in this case, if it is known that there are no outliers in X.

A third method of permutation to consider is one proposed by ter Braak (1992). Like the method of Freedman and Lane (1983), it permutes residuals. In this case, what are shuffled, however, are the residuals of the full multiple regression (Fig. 3*c*). The rationale for this is, if the null hypothesis is true, then the distribution of errors of the full model (ε' in eq. 2) should be like the distribution of errors under the null hypothesis (ε in eq. 4). The values of β_0, β_1, and β_2 are unknown, but we can obtain the least-squares estimates of these as b_0, b_1, and b_2, respectively. The residuals estimating the errors (ε') are then calculated as $R_{Y.XZ} = Y - b_0 - b_1X - b_2Z$. These residuals are permuted and, for each of the $n!$ re-orderings, a value is calculated for the squared partial correlation coefficient $(r^2_T)^\pi$ for $R^\pi_{Y.XZ}$ versus Z given X (the subscript T indicates the ter Braak (1992) method). Note once again that X and Z are not shuffled but remain in their original order. This method is sometimes called permutation "under the alternative hypothesis" or "under the full model" (ter Braak 1992; Anderson and Legendre 1999). Like the method of Freedman and Lane (1983), it assumes a linear model to calculate residuals.

As might be expected, this method of permutation can have the same kind of problem as permuting raw observations Y. Things start to go awry (i.e., Type I error does not remain at the chosen significance level), if the errors under the null hypothesis (ε) have a different kind of distribution than the errors of the full model (ε'). This can happen, for example, if the errors of the full model have a highly skewed distribution and the covariable contains an outlier (Anderson and Legendre 1999). Empirically, ter Braak's method of permutation (1992) for multiple regression gives results highly comparable with those obtained by the method of Freedman and Lane (1983). Very extreme situations need to be simulated before any destabilization occurs. Like the method of Freedman and Lane (1983), ter Braak's method (1992) is only asymptotically exact. It too must rely on estimates of regression coefficients (b_1 and b_2) to obtain residuals. If sample sizes are small ($n < 10$), then this method is not optimal.

Recommendations

In general, for tests in partial regression, the best method to use is that of Freedman and Lane (1983). It is the closest to a conceptually exact permutation test (Anderson and Robinson 2001). If sample sizes are very small ($n < 10$), it is better to use permutation of raw data, provided there are no outliers in the covariables (Anderson and Legendre 1999).

One computational advantage of ter Braak's method (1992) is for the situation when many partial regression coefficients are being tested in a single model. For example, say partial tests are to be done for the relationship between bivalve growth and each of POM, salinity, dissolved oxygen, and temperature. For each test, the other independent variables are covariables. With ter Braak's method (1992), only one set of residuals (the residuals of the full model) needs to be shuffled, regardless of which particular independent variable (or set of variables) in the model is being tested. So all the tests can be achieved by permuting one set of residuals. This differs from the situation for the method of Freedman and

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Lane (1983), which would require a new set of residuals to be calculated for each particular null hypothesis being tested.

In some situations for multiple regression, one has several repeated values of an independent variable. For example, there could have been three different temperature regimes, with several replicate observations of bivalve growth and POM in each temperature regime (i.e., X takes three repeated fixed values). In that case, an exact permutation test of Y versus Z given X could be achieved by restricting the permutations to occur only among the observations from the same temperature regime (Brown and Maritz 1982). That is, individual measurements of bivalve growth within the different temperature regimes are exchangeable (as against POM) under the null hypothesis, but they are not exchangeable across different temperature regimes. Restricted permutations are considered in more detail below for multifactorial ANOVA.

Complex designs—multifactorial analysis of variance

The method of permutation required to obtain an exact test in ANOVA is not simple when there are several factors in the design. In fact, for some terms (i.e., interaction terms), there is no exact permutation test. So, for tests of interaction, an approximate permutation test that is asymptotically exact must be used. There are some general guidelines, however, that can help in making a decision about two important aspects of permutation tests for complex designs (M.J. Anderson and C.J.F. ter Braak, unpublished data). First, one must determine what is to be permuted (i.e., what units are exchangeable under the null hypothesis). Second, one must control for other factors not being tested by either (*i*) restricting permutations (which yields an exact test) or (*ii*) permuting residuals (which yields an approximate test). If the exchangeable units are the observations, then one can also obtain an approximate test by unrestricted permutation of raw data. I consider, here, nested and factorial two-way designs, to illustrate some of the important concepts for constructing permutation tests in multifactorial ANOVA.

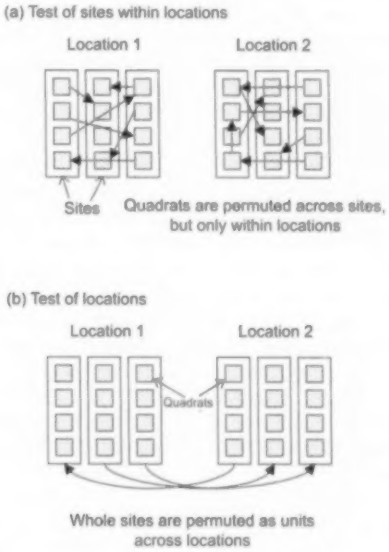
Nested (hierarchical) designs

The method of permutation used depends on the factor being tested. For an exact test of a nested factor, the permutations are done randomly across the units but are restricted to occur within each category of the higher-ranked factor in the hierarchy (Fig. 4*a*). This is a consequence of the logic of the experimental design. The categories of a nested factor are specific to each of the categories of the factor in which they are nested, so it is not logical to permute values across different categories of the upper-level factor. For example, consider a nested hierarchical design with two factors: two locations (scales of hundreds of metres) and three sites nested within each location (scales of tens of metres). The number of snails, for example, is counted within each of n = 4 replicate 1 m × 1 m quadrats at each site.

The linear model for the number of snails in quadrat k of site j within location i is

$$(5) \qquad y_{ijk} = \mu + A_i + B(A)_{j(i)} + \varepsilon_{ijk}$$

Fig. 4. Schematic diagram of units that are permuted for a test of sites (*a*) or locations (*b*) in a two-way nested ANOVA design with observations from n = 4 replicate quadrats in each of three sites nested in each of two locations.



where μ is the overall mean, A_i is the effect of location i, $B(A)_{j(i)}$ is the effect of site j within location i, and ε_{ijk} is the error associated with quadrat k in site j of location i.

For a test of differences in the numbers of snails among sites within locations, values obtained for replicate quadrats are exchangeable among the sites but they are not exchangeable across the different locations (Fig. 4*a*). Effects due to locations must be controlled in some way. In general, there are two ways of doing this. First, one can control for location differences by restricting the permutations to occur within each location. When permutations are restricted within each location, the sum of squares due to locations (SS_L) remains fixed throughout the permutations. The following is already known about the ANOVA model: (*i*) SS_T, the total sum of squares, remains constant across all permutations and (*ii*) $SS_T = SS_L + SS_{S(L)} + SS_R$, where $SS_{S(L)}$ is the sum of squares due to sites within locations and SS_R is the residual sum of squares. The F ratio for the test of sites within locations is

$$(6) \qquad F_{S(L)} = \frac{SS_{S(L)}/2(3-1)}{SS_R/6(4-1)}$$

If the null hypothesis were true, the variability due to sites within locations would be similar to the residual variation. That is, variability due to sites is exchangeable with residual variation under the null hypothesis. If SS_T and SS_L remain constant under permutation, then the shuffling strategy is "mixing" variability only between $SS_{S(L)}$ and SS_R, which gives an exact test for differences among sites.

Second, one can control for location effects in another way by obtaining the residuals, which is done by subtracting means:

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(7) $$r_{ijk}^{(B)} = y_{ijk} - \bar{y}_{i..}$$

where $\bar{y}_{i..}$ is the mean for location i. Permuting these residuals and recalculating $F_{S(L)}$ under permutation gives a good approximate test. Simulations have shown that the exact test is more powerful in this particular situation, so there is no real advantage to be gained by permuting residuals, unless using restricted permutations yields too few possible permutations for a reasonable test (M.J. Anderson and C.J.F. ter Braak, unpublished data).

Next, for the test of the higher-ranked factor (locations), it is known that components of variability associated with the nested term (sites) are incorporated into its expected mean square (Scheffé 1959). Thus, the mean square of the nested factor (sites) is the appropriate denominator mean square for the F ratio of this test. If the null hypothesis were true, then variability from site to site would be exchangeable with variability due to locations. So, for a permutation test of locations, the four replicate quadrats in any one site are kept together as a unit. These units (i.e., the different sites, of which there are six in the above design) are permuted randomly across the two locations (Fig. 4*b*). The F ratio for the test is then:

(8) $$F_L = \frac{SS_L/1}{SS_{S(L)}/2(3-1)}$$

This demonstrates the notion of exchangeable units for permutation tests in ANOVA. In general, for any term in any ANOVA model, the appropriate exchangeable units are identified by the denominator mean square in the F ratio for the test (M.J. Anderson and C.J.F. ter Braak, unpublished data). If quadrats within sites are permuted together as a unit (i.e., whole sites are permuted), then SS_R remains constant under permutation. As SS_T also remains constant under permutation, the shuffling of these units clearly exchanges variability only between SS_L and $SS_{S(L)}$. This gives an exact permutation test for differences among locations, given that significant variability may occur across sites within locations.

No approximate tests are possible here. Random permutation of raw data or of any kind of residuals across all observations (ignoring the site units) results in inflated Type I error for this test, when significant variability due to sites is present (M.J. Anderson and C.J.F. ter Braak, unpublished data).

In the above example, there are only three sites within each location, which gives $6!/[2!(3!)^2] = 10$ total possible permutations. Thus, the smallest possible P value obtainable is 0.10. This is an important consequence of the choice of experimental design on the permutation test. If given the choice, a researcher would be well advised to increase the number of categories of the nested factor (sites), rather than increasing the number of replicates (quadrats). This will give greater ability to test the higher-ranked factor in the hierarchy (locations) using permutations.

One way to increase the possible number of permutations for the test of locations is to ignore the effect of sites. This can only logically be done when variation from site to site is not statistically significant. In this case, one may then consider all quadrats to be exchangeable for the test of differences among locations. Considering the consequences of ignoring sites for the test of locations is analogous to the consideration of whether "to pool or not to pool" in traditional analyses. Thus, the general rule of thumb is not to pool (i.e., do not permute individual quadrats in the test of locations) unless the P value associated with the test of site effects is larger than 0.25 (Winer et al. 1991). If in doubt about the possible effects of sites (e.g., $0.05 < P < 0.25$), stick with the exact test (i.e., permute the sites, keeping quadrats within each site together as a unit) in the test for effects of locations. The decision about what to permute in the test of locations (i.e., quadrats or site units) when sites do not differ significantly may also depend on the severity of the consequences of making Type I versus Type II errors. The recommended criterion for the pooling of $P > 0.25$ is merely a rule of thumb (Winer et al. 1991).

In general, one must permute appropriate exchangeable units. These are identifiable by reference to the denominator mean square of the F ratio (M.J. Anderson and C.J.F. ter Braak, unpublished data).

Factorial (orthogonal) designs

Consider a two-factor experiment examining the effects of predators and the effects of food (and their possible interaction) on the numbers of snails on a rocky shore. There are, for example, areas where predators have been experimentally removed and areas where predators have not been removed (factor A). In addition, for each of these states (presence or absence of predators), there are some areas where the amount of food (algae) has been reduced and other areas where food has been left intact (factor B). Say that, in each of these combinations of factors A and B (there are four—with or without food in the presence or absence of predators), the numbers of snails are recorded in $n = 5$ replicate quadrats.

The linear ANOVA model is

(9) $$y_{ijk} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{ijk}$$

where μ is the population mean, A_i is the effect of category i of factor A, B_j is the effect of category j of factor B, AB_{ij} is the interaction effect of the ijth combination of factors A and B, and ε_{ijk} is the error associated with observation y_{ijk}.

For the test of a significant interaction ($A \times B$), there is no exact permutation test. The problem is to create a test for the interaction, controlling for the possibility of there being main effects of some kind. Restricting permutations to within categories of other factors is one method of controlling for these factors in the model. If, however, permutations were restricted to occur within each of the four combinations of predators and food (categories of factors A and B), there would be no other values of the F statistic for the test of interaction apart from the one obtained with the original data.

Thus, an approximate permutation test is necessary here. One may control for the main effects by estimating residuals. The main effects are unknown, but can be estimated by calculating the means (or, in multivariate analysis, the centroids, which consist of the means for each variable) for each group in each of the factors. Effects of the factors can then be "removed" by subtracting the appropriate mean from each observation to obtain residuals. For a single variable, let y_{ijk} be the kth observation from the ith category of factor

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A and the jth category of factor B. Then the residuals removing the effects of A and B are

$$(10) \quad r_{ijk}^{(AB)} = y_{ijk} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{\bar{y}}_{...}$$

where $\bar{y}_{i..}$ is the mean for category i of factor A, $\bar{y}_{.j.}$ is the mean for category j of factor B, and $\bar{\bar{y}}_{...}$ is the overall mean. If there were no significant interaction of these factors (i.e., the null hypothesis about interactions were true), then these residuals would be estimates of the errors associated with each sampling unit in the model (without interaction) and are iid. These residuals are thus exchangeable under the null hypothesis of no interaction and can be permuted to obtain a test. Note that it was necessary to assume an additive ANOVA model to get the residuals for this approach.

This method of permutation was described by Still and White (1981) and is the equivalent counterpart in ANOVA to the method of Freedman and Lane (1983) in partial regression. It is generally the best method to use of the approximate methods, because empirically it gives the best power and maintains Type I error for complex designs in the widest circumstances (M.J. Anderson and C.J.F. ter Braak, unpublished data). This is, however, only an asymptotically exact test, because the "true" cell means corresponding to effects of different factors are not known. Consequently, when the residuals are calculated from these, they do not correspond to the "true" errors. This can be problematic with small sample sizes, where estimates of means are not very precise. With increases in sample size, estimates of means get better, and permutation of residuals gets closer to being an exact test.

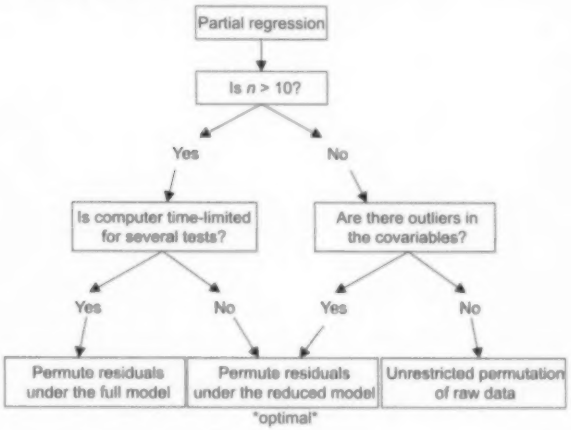
It is also possible to use the permutation method of ter Braak (1992) in the test for interaction in ANOVA. Here, the residuals that are permuted are those corresponding to the estimates of the errors for the full model, including the interaction. So these residuals are calculated as

$$(11) \quad r_{ijk}^{(\text{full})} = y_{ijk} - \bar{y}_{ij.}$$

where $\bar{y}_{ij.}$ is the mean of the n replicates in the cell corresponding to the ith category of factor A and the jth category of factor B. In practice, this method gives results that are highly comparable with those obtained using permutation of residuals under the reduced model (Anderson and Legendre 1999).

Another approach to obtain an approximate test of the interaction is simply to permute the raw data without restriction (Manly 1997). This can be used for tests in ANOVA designs where exchangeable units are the individual observations (i.e., any tests where the denominator mean square of the F ratio is the residual). Permutation of raw data will not have the same problem in ANOVA that it has when used for tests in partial regression. There are no such things as "outliers" in codes (or dummy variables) used to identify categories in the multiple regression model corresponding to an ANOVA design (e.g., Neter et al. 1996). Also, unrestricted permutation of raw data may be preferred over either method of permutation of residuals in the case of small sample sizes (Gonzalez and Manly 1998), because no estimates of means are required. However, simulations have shown that permutation of residuals does give a more powerful test (M.J. Anderson and C.J.F. ter Braak, unpublished data).

Fig. 5. Flow chart indicating appropriate permutation methods for tests of partial regression.



If the interaction is not significant, then tests of main effects are the next logical step. The objective is to obtain a permutation test for, say, the effect of factor A, while controlling for the possible effect of factor B. An exact test (in the absence of any interaction) is achieved by restricting the permutations to occur within categories of factor B. So, for example, to test for the effects of predators, one would permute values within each of the food treatments. Alternatively, permutation of residuals (under either the reduced or full model) can also be used. Note that for permutation under the full model, the residuals to be permuted are the same regardless of the term being tested. The residuals to be permuted under the reduced model depend, however, on the term being tested. For example, the residuals that would be permuted for the test of factor A, removing the effect of factor B, if any (in the absence of an interaction), are

$$(12) \quad r_{ijk}^{(A)} = y_{ijk} - \bar{y}_{.j.}$$

For testing factor B in the presence of A (without interaction), the residuals are

$$(13) \quad r_{ijk}^{(B)} = y_{ijk} - \bar{y}_{i..}$$

Simulations have demonstrated that permutation of residuals under the reduced model in this situation is more powerful than an exact test using restricted permutations (M.J. Anderson and C.J.F. ter Braak, unpublished data).

Note that the above strategies for permutation tests in factorial designs also apply to observational studies with factorial structures and to BACI (before-after, control-impact) studies.

Recommendations

Some general recommendations for practical situations are appropriate. Empirical simulations supporting these statements can be found in the unpublished data of M.J. Anderson and C.J.F. ter Braak and in Manly (1997) and Gonzalez and Manly (1998).

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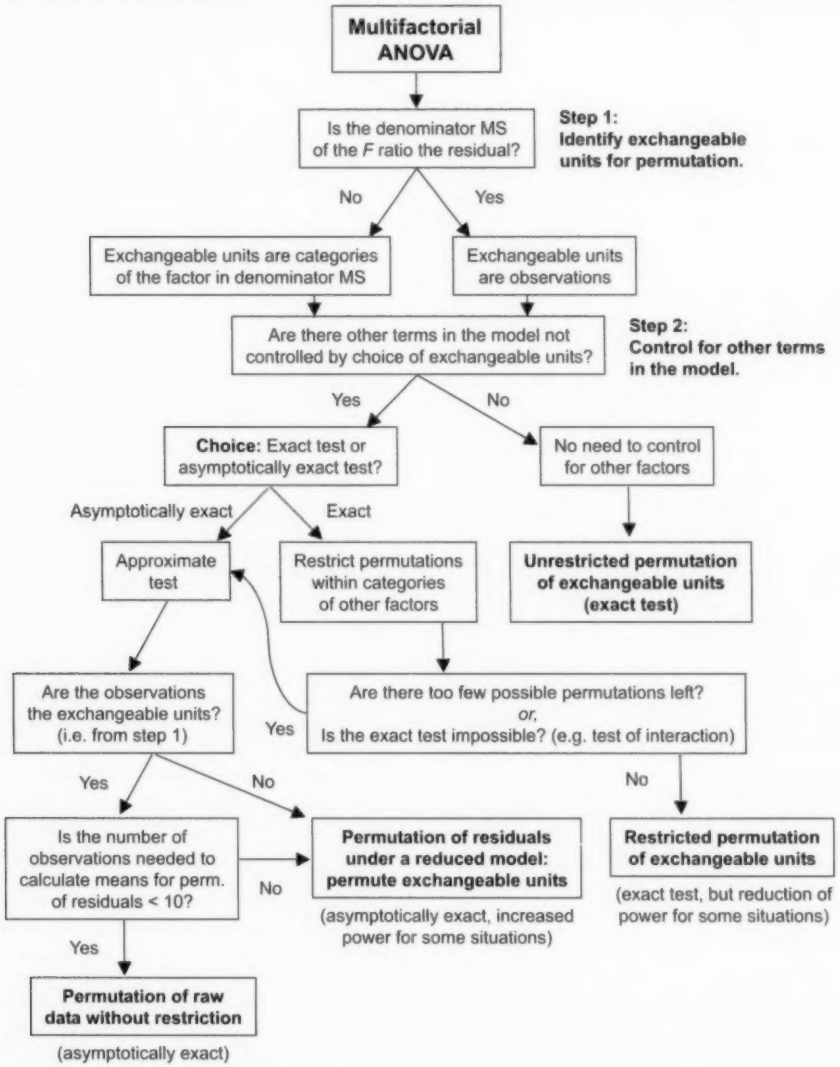
Fig. 6. Flow chart indicating appropriate permutation methods for tests of individual terms in multifactorial ANOVA.


Multifactorial ANOVA
Is the denominator MS of the F ratio the residual?
Step 1: Identify exchangeable units for permutation.
No
Yes
Exchangeable units are categories of the factor in denominator MS
Exchangeable units are observations
Are there other terms in the model not controlled by choice of exchangeable units?
Step 2: Control for other terms in the model.
Yes
No
Choice: Exact test or asymptotically exact test?
No need to control for other factors
Asymptotically exact
Exact
Approximate test
Restrict permutations within categories of other factors
Unrestricted permutation of exchangeable units (exact test)
Are the observations the exchangeable units? (i.e. from step 1)
Are there too few possible permutations left? or, Is the exact test impossible? (e.g. test of interaction)
Yes
Yes
No
No
Is the number of observations needed to calculate means for perm. of residuals < 10?
Permutation of residuals under a reduced model: permute exchangeable units
No
(asymptotically exact, increased power for some situations)
Restricted permutation of exchangeable units
(exact test, but reduction of power for some situations)
Yes
Permutation of raw data without restriction
(asymptotically exact)

The first step for any permutation test in an ANOVA context is to identify the exchangeable units. These are identifiable by reference to the expected mean squares. The denominator mean square for the F ratio of any individual term will indicate which units are exchangeable. For example, the levels of the nested factor are exchangeable for the test of the higher-ranked factor in a nested hierarchy. Similarly, if an interaction term is the denominator of a relevant F ratio, then the cells corresponding to that interaction are the exchangeable units for the test. If the denominator mean square is the residual, then individual observations are exchangeable.

The second step is to control for other factors in the model not being tested (which are not already controlled by the choice of exchangeable units). There are two ways of doing this. The first is to restrict permutations within the levels of the other factors. This gives an exact test (i.e., its Type I error is exactly equal to the a priori chosen significance level). The second is to "remove" the effects of factors not being tested by calculating residuals (i.e., subtracting means of levels of factors not under test from each observation). These residuals are then permuted and the relevant F ratio under permutation is calculated using them (rather than the original observations), accordingly. Permutation of residuals in this way yields a test that is asymptotically exact (i.e., its Type I error asymptotically approaches the a priori chosen significance level with increases in sample size). Permutation of residuals also generally yields a more powerful test than the test using restricted permutations (M.J. Anderson and C.J.F. ter Braak, unpublished data).

If exact Type I error is of extreme importance, then use an exact test. However, there are several situations where one will prefer to choose an approximate test instead: (*i*) one wishes to increase power, (*ii*) the number of possible permu-

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tations is too few with the exact test to give a reasonable *P* value, or (*iii*) the exact test is impossible (e.g., tests of interaction). In these situations, one may choose to use an approximate test such as permutation of residuals. Note that the increase in power does not come at the expense of Type I error, which is maintained for the approximate tests. Also note that permutation of residuals of certain factors in the design never allows one to avoid the issue of the choice of appropriate exchangeable units. These are fixed by the design and cannot be altered by changing the permutational approach.

Should an approximate method be chosen, similar recommendations would apply as for the situation with partial regression. Namely, permutation of residuals under the reduced model is generally to be preferred over permutation of residuals under the full model, as it comes the closest to a conceptually exact test (Anderson and Robinson 2001). Permutation of raw data can only be used if the exchangeable units are the individual observations. It is recommended only in the case of small sample sizes (i.e., as a rule of thumb, if the number of observations used to calculate means for the reduced model is less than 10; Anderson and Legendre 1999).

Discussion

In practice, it is most important that the assumptions underlying the permutation tests used are kept in mind. Since permutation tests are touted as "distribution free," they are often incorrectly construed as having "no assumptions." The general assumption is that errors are iid (see the Background and rationale for permutation tests, above) and, for any test using residuals, an additive linear model is also assumed. At the very least, one must assume that relevant units are exchangeable under some null hypothesis. The examples given here are discussed in the context of a single response variable (e.g., sizes of bivalves, numbers of snails), but the same general principles for permutation apply to tests of multivariate data in complex designs (e.g., Clarke 1993; ter Braak and Šmilauer 1998; Anderson 2001).

The recommendations given in the text are consolidated into flow charts for choosing an appropriate permutation method. These flow charts are provided on the basis of previous empirical results (Gonzalez and Manly 1998; Anderson and Legendre 1999; M.J. Anderson and C.J.F. ter Braak, unpublished data) and theoretical comparisons (Anderson and Robinson 2001), which can be consulted for further details, if required. Figure 5 provides a flow chart for partial regression and Fig. 6 provides a flow chart for analysis of variance. These are general guidelines, applicable to tests of any term in any linear multifactorial model. The logic of the experimental design and the null hypothesis being tested remain paramount in considering what units to permute and how to permute them.

Perhaps the most important consideration in the development of an appropriate permutation test is to determine what units are exchangeable under the null hypothesis. For partial regression, it is useful to write down the full model and then write down what the model would look like if the null hypothesis were true. Assuming the errors under a true null hypothesis are iid, this will indicate what residuals are exchangeable for permutation using the Freedman and Lane

Table 1. List of some computer software packages and their uses for permutation tests.

Computer package	PC or Macintosh	ANOVA or regression	Methods implemented[a]	Reference
CANOCO[b]	PC	ANOVA, regression	D, R, F, S, E	ter Braak and Šmilauer 1998
MULTIV[b]	Macintosh	ANOVA	D, S	Pillar and Orlóci 1996
NPMANOVA[b]	PC	ANOVA	D, R, F, S, E	Anderson 2001
PATN[b]	PC	ANOVA	D	Available from the Commonwealth Scientific and Industrial Research Organisation, Division of Wildlife and Ecology, Australia
PRIMER[b]	PC	ANOVA	D, S, E	Clarke 1993
The R Package[b]	Macintosh	ANOVA, regression	D, R, S	Legendre and Vaudor 1991
RT	PC	ANOVA, regression	D, F, S	Manly 1997
StatXact	PC	ANOVA, regression	D, S	Available from Cytel Software, Cambridge, Mass., U.S.A.
Various Fortran routines	PC or Macintosh	ANOVA, regression	D, S, E	Edgington 1995

[a]D, raw data permutation; R, permutation of residuals under the reduced model; F, permutation of residuals under the full model; S, restricted permutations; E, possible to exchange units other than observations.
[b]Permutation tests in these packages were primarily intended for use with multivariate data, but may be used for univariate applications as well.

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(1983) method. Although one has the option in partial regression of using permutation of raw data or the method of ter Braak (1992), the method of Freedman and Lane (1983) is to be preferred, unless sample sizes are very small ($n < 10$), in which case, permuting raw data is preferred, provided there are no outliers in the covariables (Anderson and Legendre 1999).

In the case of analysis of variance, for the exact test, one must consider (*i*) what units to permute and (*ii*) if permutations should be restricted (for an exact test) or if residuals should be permuted (for an asymptotically exact test). To determine *i*, consider the *F* ratio for the particular term being tested. What is the term identified by the denominator mean square? The categories of this factor indicate the units that are exchangeable under the null hypothesis (M.J. Anderson and C.J.F. ter Braak, unpublished data). For example, if the denominator mean square is the residual, then observations themselves can be permuted. If the denominator mean square is an interaction term $A \times B$, then *ab* cells need to be permuted as units. To determine *ii*, recall that the total sum of squares remains constant across all permutations. For an exact test, permutations must be restricted to occur within categories of all other factors in the model not being tested, if their variability is not already controlled by the choice of units in *i*. For an approximate test, one can permute residuals rather than restricting permutations. Note, however, that this does not alter the fact that the correct exchangeable units must still be used.

When an exact test cannot be done (e.g., tests of interaction) or there are too few possible permutations to give enough power with an exact test, then permutation of residuals under the reduced model (in the manner of Freedman and Lane (1983)) is highly recommended. Permuting residuals in ANOVA corresponds to permuting units after subtracting means corresponding to categories of particular factors (Still and White 1981). Use unrestricted permutation of raw data if sample sizes are very small. As a general rule of thumb, if the means (averages) needed to calculate residuals for the Freedman and Lane (1983) method are obtained by fewer than 10 observations, then use unrestricted permutation of raw data (Gonzalez and Manly 1998; Anderson and Legendre 1999).

The recommendations given here provide the basis for constructing exact permutation tests, where possible, or for choosing an optimal approximate permutation test. More detailed statistical information and empirical and theoretical comparisons of these methods can be found elsewhere (Anderson and Legendre 1999; Anderson and Robinson 2001; M.J. Anderson and C.J.F. ter Braak, unpublished data).

A note on computer software

It is always problematic to mention much in the way of available computer software, for as soon as such information is printed, it is out of date. However, Table 1 contains a list, which is by no means intended to be exhaustive, of the available software packages known to me that are capable of performing various permutation procedures referred to in the text. I have not included in this list any of the major statistical software packages, such as SAS, SPSS, S-PLUS, or Minitab, which can be used to perform various permutation methods by programming such procedures using their built-in languages. For programming various methods in FORTRAN, I used the GGPER routine from the International Mathematical and Statistical Library (IMSL) and I have found the books by Edgington (1995) and Manly (1997) to be particularly helpful.

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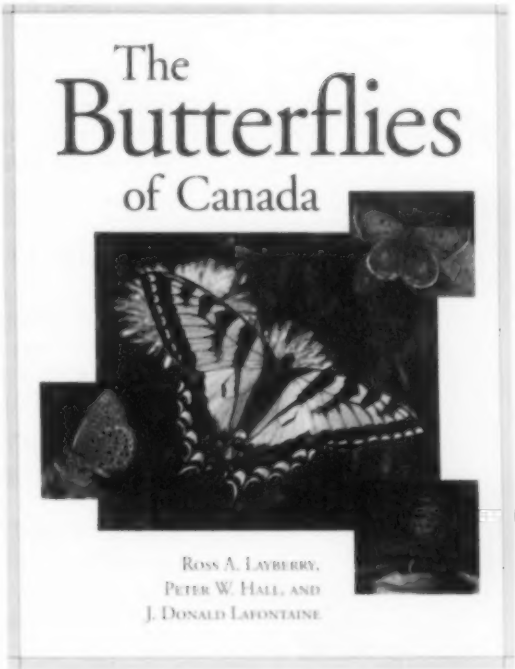

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